Clariant Corporation
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November 27, 2006

Ref: EPA-HQ-OPPT-2005-0055 RIN 2070-AB11
Clariant Corp. Submission of Health and Safety Studies for HPV Orphan Chemicals

VIA COURIER

US Environmental Protection Agency
OPPT Document Control Office
EPA East Room 6428
1201 Constitution Ave. NW
Washington, DC 20004-3302
Attn: 8(d) Health and Safety Reporting Rule (Notification/Reporting)

CONTAIN NO CBI

Phone 202 564-8930

Dear Sir:

Clariant Corp. is submitting copies of unpublished Health and Safety Studies with robust summaries for Ethanesulfonic acid, 2-[methyl[(9Z)-1-oxo-9-octadecenyl]amino]-,sodium salt, CAS No. 137-20-2. This chemical is imported via our US headquarters site at:

Clariant Corp.
4000 Monroe Road
Charlotte, NC 28205

If you have any questions, please call me at the numbers listed above

Sincerely,

Terry L. Wells
Product Safety Manager
Clariant Corp.
Functional Chemicals

Enclosures

Acute Toxicity to Pseudomonas putida

Test Substance: Ethanesulfonic acid, 2-[methyl[(9Z)-1-oxo-9-octadecenyl]amino]-,sodium salt, CAS No. 137-20-2.
Test Substance Purity/Composition: >95%
Method : DIN 38412 Teil 8 from Nov 1987
Year Study Performed 1989
Species/In Vitro System Pseudomonas putida
GLP: Not indicated
Test Concentrations 170, 380, 600, 875 mg/L
Nominal and Measured Concentrations Not indicated
pH Value 7.0 (adjusted)
Test Temperature 21-24 C
Test Results **EC0 = 170 mg/L**
EC50 =380-600 mg/L
EC100= 875 mg/L
Reference – Hoechst V 89-274-B

Hoechst

An ATA-TH-TVS
C655

An SITOX, C 769, [SITOX Nr.: 484]

Hoechst Aktiengesellschaft
Abteilung Umweltschutz
Postfach 800320
D-6230 Frankfurt am Main 80

Datum 1. Sep. 1989

Untersuchung auf Bakterienschädlichkeit: Zellvermehrungs-Hemmtest

Berichtsnummer: V 89-274-B

Probe: Arkopon T Teig extra

Die Bestimmung der Bakterienschädlichkeit erfolgte im Zellvermehrungs-Hemmtest (Wachstums-Hemmtest) nach Brinkmann und Kühn unter Verwendung von *Pseudomonas putida* MIGULA, Stamm Berlin 33/2 (DSM 50026).

Die Untersuchung erfolgte anhand des Normentwurfs DIN 38412 Teil 8 vom November 1987:
Bestimmung der Hemmwirkung von Wasserinhaltsstoffen auf Bakterien (Pseudomonas-Zellvermehrungshemmtest) (L8).

Ergebnis:

Bei gesättigter Lösung unbekannter Konzentration:

EC_0 = 170 mg/l Verdünnung 1 : _____

EC_{50} = 380-600 mg/l 1 : _____

EC_{100} = 875 mg/l 1 : _____

Anmerkungen: Stammlösung: 1000 mg/l, pH = 6,9 (nicht korrigiert)

Erläuterungen: EC0 ist die höchste Konzentration bzw. kleinste Verdünnungsstufe, bei der keine signifikante Hemmwirkung eintritt (Grenzwert 20 %). EC50 ist die Konzentration oder Verdünnung, bei der die Schädigung 50% beträgt. EC100 ist die niedrigste Konzentration bzw. größte Verdünnungsstufe, bei der die Hemmung 90 % überschreitet. Die EC-Werte von Produkten werden durch halblogarithmische Regression ermittelt.

Dr. Voelskow

Prüfberichte: ökologische Untersuchungen

Hoechst

An Herrn Baumann — C655
Auftraggeber — Gebäude

ATA TH-TVS — Hoe
Abteilung — Werk

An Herrn
Kontaktstelle für Umweltschutz im Geschäftsbereich — Gebäude

GB-E
Abteilung — Werk

An Herrn Baum, SITOX — C 769
Dokumentation — Gebäude

Hoechst Aktiengesellschaft
Abteilung Umweltschutz
Postfach 800320
D-6230 Frankfurt am Main 80

Datum 8. Sep. 1989

Berichtsstammnummer: V-89- 274

Probe: Arkopon T Teig extra

Charge: E 0 1 112 195

Reinheit/Konzentration:

CAS-Nr.: **Analyse (Nr., Datum):**

Probevorbereitung:

(zutreffende Angaben ausgefüllt oder angekreuzt)

☒ Produktprobe: Einwaage zur Herstellung der Stammlösung = 5 g/l.

☐ Abwasserprobe: Vorverdünnung (incl. pH-Einstellung) = 1 :

Der pH-Wert wurde mit _____ von 7,0 auf / eingestellt

und der Ansatz 24 Std. bei 21 °C gerührt; pH-Wert danach: /;

pH ggf. wieder korrigiert: auf /. Das Testgut war anschließend:

☒ klar gelöst; ☐ kolloidal gelöst/suspendiert/emulgiert; ☐ teilweise gelöst.

☒ Der Ansatz ☐ Der filtrierte Ansatz wurde als Stammlösung verwendet.

Anmerkung: Bei Mischpräparaten wird durch die Filtration ein Extrakt mit eventuell stark veränderter Zusammensetzung gewonnen, bei Reinsubstanzen eine gesättigte Lösung.

Die Angaben zum Ansatz gelten ausschließlich für Teilberichte im Anhang dieses Blattes.

Die ökologischen Untersuchungen mit genannter Testsubstanz umfassen folgende Teilberichte:

		Teilbericht im Anhang Seite:
☒	Summenparameter DOC, CSB	2
☐	Grundsätzliche biologische Abbaubarkeit im Zahn-Wellens-Test	
☐	Leichte biologische Abbaubarkeit	
☐		
☒	Hemmwirkung gegenüber Bakterien folgt später	
☐	Akute Toxizität gegenüber Daphnien	
☐		
☐		

Prüfbericht: Summenparameter

hoechst

Berichtsnummer: (Stammnr. - Versuchskennung)

V-89- 274 -S

Spezifikation der Probe: s. Seite 1.

Hoechst Aktiengesellschaft
Abteilung Umweltschutz
Postfach 800320
D-6230 Frankfurt am Main 80

Seite: 2 (2)

Datum 8. Sep. 1989

Bestimmung der Summenparameter:

SITOX Nr.	Parameter	Testnorm	gemessene Werte in der Stammlösung: (1)	gemessene Werte pro g Substanz, Berechnung: (2)	berechnete, theoretische Werte: (3)
451	DOC	DIN 38 409 Teil 3	1940 mgC/l	388 mgC/g	mgC/g
452	CSB	EEC 84/449 und DIN 38 409 Teil 41	6.145 mgO_2/l	1229 mgO_2/g	mgO_2/g

Erläuterungen:

(1) Bei Produktprüfung: Stammlösung gemäß Angaben auf Seite 1 dieses Berichtes, bei Abwasserprüfung: Werte auf die unverdünnte Abwasserprobe hochgerechnet.

(2) Die Methode der Ermittlung der Werte pro Gramm ist angekreuzt:

☒ Bei Lösungen und stabilen Dispersionen definierter Einwaage erfolgt die Berechnung der Angaben pro Gramm aus den Meßwerten pro Liter der Stammlösung.

☐ Der CSB schwerlöslicher Reinsubstanzen wird indirekt über den gemessenen CSB und den gemessenen DOC der gesättigten Lösung mit Hilfe des berechneten DOC/g ermittelt. Ein gemessener DOC/g kann nicht angegeben werden.

☐ Für teilweise gelöste Mischpräparate können keine Werte pro g berechnet werden, da die Konzentrationen der Einzelkomponenten nach der Filtration nicht mehr feststellbar sind. Der CSB solcher Produkte wird mit Direkteinwaage (ca. 1 - 2 mg) in das Reaktionsgefäß bestimmt. Ein gemessener DOC/g kann nicht angegeben werden.

☐ Filtrierte Dispersionen werden für die CSB-Bestimmung wie schwerlösliche Substanzen behandelt, wenn die Berechnung des DOC/g möglich ist, sonst wie teilweise gelöste Mischpräparate. Das entsprechende Feld für die gewählte Methode ist oben zusätzlich angekreuzt. (Die Abtrennung gröberer Partikel oder Tropfchen durch Filtration ist für die DOC-Bestimmung und ggf. auch für weitere Versuche erforderlich).

(3) Der DOC- und ein theoretischer CSB-Wert für 100 % Oxidierbarkeit können aus der Summenformel (sofern vorliegend) berechnet werden. Sie werden ermittelt, wenn sie als Grundlage anderer Berechnungen oder für die Auswertung bestimmter ökologischer Versuche erforderlich sind, sowie beim Verdacht auf größere Abweichungen von gemessenen, auf ein Gramm bezogenen Werten.

Anmerkung: Bei mehrfacher Messung von Summenparametern in Ansätzen für verschiedene Teilversuchen können die Werte im Rahmen der Fehlerbreite der Analysenmethoden unterschiedlich ausfallen. Bei der Herstellung gesättigter oder sonstiger filtrierter Lösungen gemäß der auf Seite 1 angegebenen Standardmethode sind größere Abweichungen möglich.

Dr. Voelskow

Ready Biodegradability Modified Sturm Test

Test Substance: Ethanesulfonic acid, 2-[methyl[(9Z)-1-oxo-9-octadecenyl]amino]-,sodium salt, CAS No. 137-20-2.
Test Substance Purity/Composition: 62.7%
Method/Guideline Description OECD 301 B Guideline/CO2 Evolution Test for Testing of Chemicals adopted July 17, 1992
GLP Yes
Concentration Value 30 mg/L
Time in Days 28 days
Biodegradation Value 80% after 28 days
Biodegradation Value 10% after 5 days
Temperature 20-24C
Incubation Condition Aerobic
Inoculum Type Inoculum of the aqueous phase of non adapted activated sludge
Pre-Exposure Remarks The activated sludge was maintained in an aerobic condition by aeration for four hours and then homogenized with a mixer. The sludge was filtered and the filtrate was subsequently used to initiate inoculation
Theoretical Carbon DiOxide 1.42 mg CO2/mg test item
Control Substance Remarks Sodium acietate35 mg/L
Results Remarks The 10% level was reached after an adaptation phase of 5 days. The 60% level was reached after 22 days and the biodegradation came to a maximum of 80% after 28 days. The test item is readily biodegradable.
Study/Method - Biodegradation
Key Study Sponsor Indicator
Year Study Performed 2004
Method/Guideline Followed Yes
Deviations from Method/Guideline None
Study Reference Dr. U. Noack Laboratorien Study No. AST97821
Reliability/Data Quality
Reliability
Reliability Remarks

Hostapon TPHC

Ready Biodegradability
Modified Sturm Test

acc to OECD 301 B Guideline/CO_2 Evolution Test
for Testing of Chemicals (adopted July 17, 1992)

Sponsor

CLARIANT GMBH
Functional Chemicals (FUN)
Regulatory & Quality Affairs, C 655
D-65926 Frankfurt

Author

Silke Fiebig

Testing Facility

DR.U.NOACK-LABORATORIEN
Käthe-Paulus-Straße 1
D-31157 Sarstedt

Laboratory Project ID

Project-No. 040803CH
Study-No. AST97821
Study No. of the Study Plan: AST9782-

Date

24. Jan. 2005

Statement of GLP Compliance

Title	Hostapon TPHC Ready Biodegradability, Modified Sturm Test
Guideline	OECD 301 B / CO_2 Evolution Test for Testing of Chemicals (adopted 1992-07-17)
Test Item	Hostapon TPHC (Batch number: DEBD007684)
Testing Facility	DR.U NOACK-LABORATORIEN Käthe-Paulus-Str.1, D-31157 Sarstedt Phone: (+49) 050 66 / 706 70, Fax: (+49) 05066 / 706 789 E-mail: info@noack-lab.de
Deviations from GLP Principles	None

We declare that this study was conducted and reported in compliance with the present OECD, EC and German principles of Good Laboratory Practice, except deviations mentioned above.

24.1.95
(Date)

S. Fieb
(Silke Fiebig, Study Director)

(Date)

(Dr. Udo Noack, Head of Testing Facility)

Statement of the Quality Assurance Unit

Title	Hostapon TPHC Ready Biodegradability, Modified Sturm Test
Guideline	OECD 301 B / CO_2 Evolution Test for Testing of Chemicals (adopted 1992-07-17)
Test Item	Hostapon TPHC (Batch number. DEBD007684)
Study Director	Silke Fiebig

The study was verified as follows:

inspection	date of inspection	date of report
study plan	2004-10-22	2004-10-25
study based	2004-11-03	2004-11-03
	2004-11-26	2004-11-26
report	2004-12-06	2004-12-08
	2004-12-10	2004-12-10

The reported results accurately and completely reflect the raw data of the study. Also methods, procedures, and observations are accurately and completely described in the report.

The accordance of the study with its study plan and the principles of Good Laboratory Practice is guaranteed.

24.1.05

Gudrun Mohrmann-Kalabokidis

Personnel Involved

Study Director	:	Silke Fiebig (Engineer, Biotechnologist)
Deputy	:	Martina Noack (Biologist)
Technical Staff	:	Nadine Mendel Karin Ruthenberg
Quality Assurance Unit:		Gudrun Möhrmann-Kalabokidis (Biologist)
Head of the Testing Facility	:	Dr. Udo Noack (Biologist)

Table of Content

List of Tables and Figures

1 Summary

The ready biodegradability was determined with a non adapted activated sludge for the test item **Hostapon TPHC** (batch no.: DEBD007684) over a test period of 28 days in the Modified Sturm Test The study was conducted from 2004-10-28 to 2004-11-26 according to OECD 301 B / CO_2 evolution test at DR.U.NOACK-LABORATORIEN. The test item was tested with a concentration of 30 mg/L in duplicates, corresponding to a carbon content (TOC) of 11.6 mgC/L in the test vessels. The biodegradation of the test item was followed by titrimetric analyses of the quantity of CO_2 produced by the respiration of bacteria The degradation was finished on day 28 by acidification, the last titration was made on day 29, after the soluble CO_2 was turned out over a period of 24 h. The percentage CO_2 production was calculated in relation to the theoretical CO_2 ($ThCO_2$) of the test item The biodegradation was calculated for each titration time

To check the activity of the test system sodium acetate was used as functional control. The percentage degradation of the functional control reached the pass level of 60 % after 8 days. In the toxicity control containing both test and reference item a biodegradation rate of 47 % occurred within 14 days and came to a maximum of 70 % after 28 days. The biodegradation of the reference item was not inhibited by the test item in the toxicity control.

The biodegradation of the test item is shown graphically in figure 1 in comparison to the readily degradable functional control and the toxicity control. The 10 % level (beginning of biodegradation) was reached after an adaptation phase of 5 days. The pass level of 60 % was reached after 22 days and the biodegradation came to a maximum of 80 % after 28 days.

The validity criteria according to the guideline are fulfilled.

The test item must be regarded to be

readily biodegradable.

Table 1 **Biodegradation of the Test Item Hostapon TPHC in Comparison to the Functional Control and Toxicity Control**

	Biodegradation (%) study day [d]			
	6	14	21	28
test item, 1st replicate 30 mg/L	23	43	57	82
test item, 2nd replicate 30 mg/L	23	49	61	78
functional control 35 mg/L	48	71	88	100
toxicity control 30 mg/L test item + 35 mg/L reference item	21	47	56	70

2 Characterisation Data of the Test Item

TEST ITEM	Hostapon TPHC
Batch Number	DEBD007684
CAS No.	137-20-2
Chemical characterisation	Fatty acid methyl tauride, sodium salt
Purity	62 7 % (difference to 100 %: sodium chloride)
Appearance	Yellowish powder
Water solubility	150 g/L (20 °C)
pH value	7.5 (1 % a.i. in water, method DIN EN 1262)
TOC	36.6 %
Expiry date	2006-03-31
Recommended storage	Room temperature (20°C)
Storage at test facility	Room temperature, protected from moisture and light
Retention	At least 1 g has been retained.
Identification parameter at test facility	Name, batch number, state, consistency and colour

The test item and the information concerning the test item were provided by the sponsor.
TOC determined at testing facility in a preliminary test (non GLP)

3 Method

GUIDELINE	OECD 301 B / CO_2 evolution test (adopted July 17, 1992)
TYPE AND PURPOSE OF THE STUDY	Study of ready biodegradability over the test period of 28 days with a non-adapted activated sludge to determine the rate of biodegradation in %.
TEST SYSTEM	*Inoculum* of the aqueous phase of non adapted activated sludge
Reasons for the selection of the study system	*Activated sludge* from the sewage plant at Hildesheim is well suited as it comprises mostly municipal sewage and hardly industrial chemical waste.
Source	Municipal sewage treatment plant, D-31137 Hildesheim
Pretreatment	The activated sludge was maintained in an aerobic condition by aeration for four hours and then homogenized with a mixer The sludge was filtered and the filtrate (30 mL) was subsequently used to initiate inoculation.
Colony forming units of the inoculum	10^7 - 10^8 CFU/L
Colony forming units in the test vessels	10^5 - 10^6 CFU/L
FUNCTIONAL CONTROL	Sodium acetate, puriss. (FLUKA)
CAS No.	127-09-3
Batch	454025/1
Purity	100.9 %
Expiry date	2006-01-08
Replicates	Single
Test concentration	35 mg/L
$ThCO_2$	1.07 mg CO_2 /mg
ThTOC	0.29 mg C/mg
Carbon content in the vessel	10 2 mg C/L

TEST ITEM	Hostapon TPHC
Replicates	Duplicates
Test concentration	30 mg/L
TOC	38.6 %
$ThCO_2$	1.42 mg CO_2/mg test item
Carbon content in the vessel	11 6 mg C/L
TOXICITY CONTROL	Test item and reference item in test concentration and inoculum
Replicates	Single
CONTROL	Nutrient solution and inoculum
Replicates	Duplicates
PROCEDURE	
Duration	28 d
Frequency and duration of the application	Once per setting up over 28 d
Test vessels	5000 mL, brown glass
Volume of the test medium	3000 mL
Test medium	Mineral nutrient solution acc. to OECD 301 B/CO_2 Evolution Test
TYPE AND FREQUENCY OF MEASUREMENTS	The room temperature was measured continuously by a thermohygrograph. Determination of CO_2 was carried out by titration subsequent to complete adsorption of the released CO_2 in a basic solution. Backtitration of the residual $Ba(OH)_2$ with 0.05 N HCl was carried out three times a week during the first ten days and thereafter twice weekly. On day 28 the pH-value of all solutions was measured prior to acidification.
Equipment	pH-Meter, Multilab 340i, Wtw Thermohygrograph, type 3.015/3 K, fabr.-no. 9003146 Flow meter, KROHNE DUISBURG TYP DK 800 PV

COURSE OF THE STUDY

The concentration of the test item and the theoretical CO_2 production ($ThCO_2$) were calculated based on the determined carbon content of the test item.

The following test solutions were prepared in 5 L brown glass bottles as incubation vessels:

- two for the test item concentration (P_1, P_2)
- one for the reference item (R_1)
- two for the inoculum control (C_1, C_2)
- one for the toxicity control (T_1)

The necessary amounts of aqua bidest , nutrient media and inoculum were placed in each of the incubation vessel. The vessels were connected to the system for the production of CO_2 free air and aerated for 24 h.
After 24 h the CO_2 adsorption vessels were connected to the air outlets of the incubation vessels via a series of 3 gas wash bottles.

The test and reference item were weighed in and transferred into the incubation vessels, the vessels made up to 3 L with CO_2 free aqua bidest. and connected to the system for the production of CO_2 free air. Incubation took place in a temperature range of 20 - 24 °C. All vessels were stirred continuously throughout the test

On day 28 1 mL 37 % HCl was added to each of the vessels. Aeration was continued for further 24 h and on day 29 the quantity of CO_2 released in the last two gas wash bottles was determined

VALIDITY CRITERIA

The percentage degradation of the functional control must reach the pass level of 60 % by day 14.

The total CO_2 evolution in the control at the end of the test should be lower than 40 mg/L and not exceed 70 mg/L.

The difference of extremes of replicate values of removal of the test item at the end of the test or at the plateau as appropriate must be less than 20 %.

The percentage degradation of the toxicity control must reach the pass level of 35 % by day 14. If the degradation is lower than 35 % the test item must be assumed to be inhibitory and the study must be repeated with a lower test concentration.

EVALUATION

The theoretical production of carbon dioxide ($ThCO_2$) of the test item and functional control is calculated by the carbon content (1) and the sum formula (2), respectively

$$ThCO_2\ [mgCO_2/mg] = 3.67 \cdot TOC\ [mgC/mg] \quad (1)$$

$$ThCO_2\ [mgCO_2/mg] = \frac{C - Atoms \cdot molecular\ weight\ of\ CO_2}{molecular\ weight\ of\ test\ or\ reference\ item} \quad (2)$$

The produced CO_2 was calculated as follows:

$$1\ mL\ HCl\ (c = 0.05\ mol/L) = 1.1\ mg\ CO_2 \quad (3)$$

The net amount of CO_2 produced is calculated by correcting the results of the test item and functional control for endogenous CO_2 production of the control groups.

The biodegradation is calculated from the ratio theoretical CO_2 production to net CO_2 production acc. to the following equation (4).

$$Degradation\ [\%] = \frac{net\ CO_2 \cdot 100}{ThCO_2\ [mgCO_2/3L]} \quad (4)$$

The biodegradation of the test item in comparison to the readily biodegradable functional control and toxicity control is shown graphically (see figure 1).

DATES

Study initiation	2004-10-25
Experimental starting	2004-10-28
Experimental completion	2004-11-26
Study completion	Please refer to page 1

DEVIATIONS FROM THE GUIDELINE

None

DEVIATIONS FROM THE STUDY PLAN

None

ARCHIVING

The following will be retained in the archive of the test facility for the period as specified in the operative national GLP regulations:

- all raw data
- study plan
- final report
- all records performed by the quality assurance programme including master schedules
- samples of test and reference items

Microfilms will be retained in a safe-deposit by Volksbank Sarstedt, D-31157 Sarstedt.

4 Results

4.1 Carbon Content of the Test item

Based on the carbon content a $ThCO_2$ of 1.42 mg CO_2/mg test item was calculated. A test concentration of 30 mg/L, corresponding to a carbon content of 11.6 mgC/L in the test vessels was selected.

4.2 CO_2-Production and Biodegradation

The total amount of CO_2 produced in 28 days was analysed by titration in 12 measurements. The 28 d-values are shown in comparison to the readily degradable functional control in summarized form in table 2.
The results of gross and net CO_2 production and biodegradation of each measurement are given in the tables 3 and 4

Table 2 **CO_2-Production and Biodegradation after 28 Days**

CO_2-production after 28 d	control mv	functional control 35 mg/L	test item 30 mg/L No.1	test item 30 mg/L No. 2	toxicity control 35 + 30 mg/L
gross [mg/3 L]	137 9	263 8	242 5	237.7	305 1
[mg/L]	46 0	87 9	80.8	79.2	101 7
net [mg/3 L]	–	125.9	104.6	99 8	167 2
[mg/L]	–	42.0	34.9	33 3	55.7
theor [mg/3 L]	–	112.4	127 8	127.8	240 2
[mg/L]	–	37.5	42 6	42.6	80 1
Degradation [%] after 28 d	–	100	82	78	70

mv = mean value

In the **control** a maximum of 46.0 mg CO_2 /L was formed after 28 days (validity criterion: < 70 mg CO_2 /L after 28 days).

The adaptation phase of the **functional control** changes after 2 days into the degradation phase (degradation ≥ 10 %) The course of the degradation phase is rapid and reaches a degradation rate > 60 % on day 8. The validity criterion degradation ≥ 60 % after 14 d is fulfilled.
In the **toxicity control** 47 % biodegradation occurred within 14 days and came to a maximum of 70 % after 28 days. The biodegradation of the reference item was not inhibited by the test item.

The biodegradation of the test item is shown graphically in figure 1 in comparison to the readily degradable functional control and the toxicity control.
The 10 % level (beginning of biodegradation) was reached after an adaptation phase of 5 days. The pass level of 60 % was reached after 22 days and the biodegradation came to a maximum of 80 % after 28 days.

The test item must be regarded to be **readily biodegradable.**

Table 3. ***CO_2-Production and* Biodegradation for all Determination Points in the Control, Functional Control and Toxicity Control Samples**

study day	date	control	functional control 35 mg/L			toxicity control 35 mg/L reference item + 30 mg/L test item		
		[mg CO_2/3L]	[mg CO_2/3L]		degr.	[mg CO_2/3L]		degr
		mv	gross	net	[%]	gross	net	[%]
1	29 10	4.6	8 9	4 3	4	0.7	-3 9	0
4	01.11.	18.0	51.0	33 0	29	34.4	16.4	7
6	03.11.	28 2	82.6	54 4	48	79 5	51.3	21
8	05 11	40 0	109 8	69.8	62	118 3	78.3	33
11	08.11	54 0	127 8	73 8	66	150 0	96 0	40
14	11.11.	68 2	148.4	80 2	71	180.5	112 3	47
18	15 11	85 0	176 0	91 0	81	207 9	122 9	51
21	18.11	[illegible]	[illegible]	88 8	[illegible]	[illegible]	[illegible]	[illegible]
25	22 11.	113 2	222.1	108 9	97	256.5	143.3	60
28	25 11.	127.5	243.4	115.8	100	284 8	157.2	65
28*	26 11.	137.9	263.8	125.9	100	305.1	167.2	70

* results of the last two gas wash bottles
degr. = degradation mv = mean value

Table 4. **CO_2-Production and Biodegradation for all Determination Points in the Control and Test Item Samples**

study day	date	control	test item 30 mg/L					
			replicate 1			replicate 2		
		[mg CO_2/3L]	[mg CO_2/3L]		degr.	[mg CO_2/3L]		degr.
		mv	gross	net	[%]	gross	net	[%]
1	29 10	4 6	0 4	-4.2	0	0.6	-4.0	0
4	01 11.	18.0	17.8	-0 2	0	23.8	5 8	5
6	03.11.	28.2	58.1	29.9	23	57.7	29.5	23
8	05.11	40 0	86.3	46 3	36	82 8	42 6	33
11	08.11.	54.0	103 6	49 6	39	105.7	51.7	40
14	11 11.	68 2	123.0	54 8	43	130 9	62.7	49
18	15 11.	85 0	149.5	64 5	50	158 6	73.6	58
21	18.11	97.9	170.7	72.8	57	175.8	77 9	61
25	22.11.	113.2	199 4	86 2	67	201.1	87.9	69
28	25.11.	127 6	225.4	97.8	77	224 4	96.8	76
29*	26.11.	137 9	242 5	104.6	82	237.7	99.8	78

degr = degradation mv = mean value
* results of last two gas wash bottles

4.3 Water Parameter

On day 28 (2004-11-25) the pH-value of all solutions was measured prior to acidification. The results are given in table 5

Table 5. **pH-Values on Day 28**

control		functional control	test item		toxicity control
No. 1	No 2		No 1	No. 2	
7.60	7 59	7 92	7 60	7 56	7.67

5 Validity Criteria

The study was performed according to OECD 301 B / CO_2 Evolution Test and GLP Guidelines. The validity criteria were fulfilled according to the guideline:

- The total CO_2 evolution in the control at the end of the test was 46.0 mg/L.
- The degradation of the functional control reached the pass level of ≥ 60 % by day 14.
- The degradation of the toxicity control reached the pass level of 35 % after 14 days.
- The difference of extremes of replicate values of removal of the test item at the end of the test or at the plateau as appropriate was less than 20 %.

6 Literature

(1) OECD-Guideline No. 301 for Testing of Chemicals, adopted 1992-07-17

(2) OECD-Guideline No. 301 B for Testing of Chemicals, adopted 1992-07-17

(3) Regulation (EC) No. 648/2004 on Detergents

7 Graph

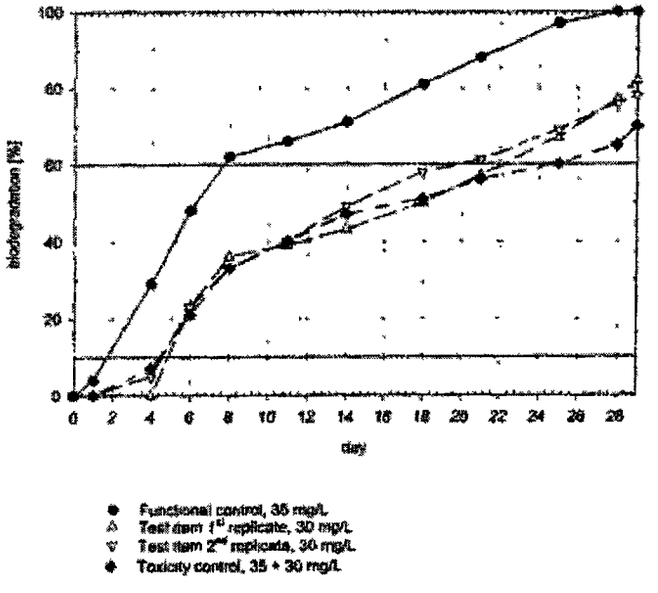

Figure 1 **Biodegradation over a period of 28 days acc. to OECD 301 B**

8 Certificate of Analysis of Test Item

Clariant GmbH
Division Pigments Chemicals
RQA, Höchst, D562
Tel. 069 / 305-85586
Fax 069 / 305-3141

Clariant

Inspection certificate Date: 17.08.2004
according to EN10204-3.1B Page: 1 / 1

Our consignment
Material : HOSTAPON TPHC/600kg à 25kg Bags i.Cdb.C
Material-no. : 10252920390
Batch No. : DEBD007684

On the batch, of which the consignment is a part, the following values were determined. They conform to the agreed product specification

Inspection characteristic/-method	Specification	Result
Active substance (M=423 g/mol) ISO 2271	60,0 - 65,0	62,7 %
pH-value (1%AS in H2O) DIN EN 1262	7,0 - 8,0	7,5
Water content Karl Fischer DIN 51777	<= 1,00	0,56 %

The above particulars do not release the customer from the obligation to carry out a inspection of goods received.

Wehner (plant surveyor)

This report is not to be signed.

9 GLP-Certificate of DR.U.NOACK-LABORATORIEN

Niedersächsisches Umweltministerium

Gute Laborpraxis/Good Laboratory Practice

GLP-Bescheinigung/Statement of GLP Compliance

(gemäß/according to § 19 b Abs 1 Chemikaliengesetz)

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw Richtlinie 88/320/EG wurde durchgeführt in

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 88/320/EEC at:

X Prüfeinrichtung / Test facility ☐ Prüfstandort / Test site

Dr. U. NOACK LABORATORIUM
für angewandte Biologie
Käthe-Paulus-Str 1
D-31157 Sarstedt

Dr. U. NOACK LABORATORY
of applied biology
Käthe-Paulus-Str 1
D-31157 Sarstedt

(Unverwechselbare Bezeichnung und Adresse/Unequivocal name and address)

Prüfungen nach Kategorien/Areas of Expertise

(gemäß/according Chem/AnV-GLP für 5.3/OECD guidance)

1 - Prüfungen zur Bestimmung der physikalisch-chemischen Eigenschaften und Gehaltsbestimmungen
3 - Prüfungen zur Bestimmung der erbgutverändernden Eigenschaften (in vitro und in vivo)
4 - Umwelttoxikologische Prüfungen zu Auswirkungen auf aquatische und terrestrische Organismen
5 - Prüfungen zum Verhalten im Boden, im Wasser und in der Luft, Prüfungen zur Bioakkumulation und zur Metabolisierung
6 - Prüfungen zur Bestimmung von Rückständen
7 - Prüfungen zur Bestimmung der Auswirkungen auf Mesokosmen und natürliche Ökosysteme

Datum der Inspektion / Date of Inspection
(Tag Monat Jahr / day month year)
25. - 27. Juni 2001 / June 25th - 27th, 2001
29. August und 21. September 2001 / August 29th and September 21st, 2001

Die genannte Prüfeinrichtung/Der genannte Prüfstandort befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht

The above mentioned test facility / test-site is included in the national GLP Compliance Programme and is inspected on a regular basis.

Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung/diesem Prüfstandort die oben genannten Prüfungen unter Einhaltung der GLP-Grundsätze durchgeführt werden können

Based on the inspection report it can be confirmed, that this test facility / test-site is able to conduct the aforementioned studies in compliance with the Principles of GLP.

Niedersächsisches Umweltministerium
Referat 33
Archivstraße 2
30169 Hannover

11 März 2002
Im Auftrag
Dr Brandt

Robust Summary

Test Item	**Hostapon TPHC**
Batch	DEBD007684
Purity	62.7 % (difference to 100 %: sodium chloride)
CAS No.	137-20-2
Chemical characterisation	Fatty acid methyl tauride, sodium salt Remarks: none
Testing Facility	DR.U.NOACK-LABORATORIEN Kathe-Paulus-Str. 1, D-31157 Sarstedt Tel. +49(0)5066 70670, email. info@noack-lab.de
Author	Silke Fiebig
Report issued	2005-01-24
Method	
Guideline followed	OECD 301 B / CO_2 evolution test (adopted 1992-07-17)
Type	Aerobic [X] Anaerobic [ ]
GLP	Yes [X] No [ ]
Year	2004
Contact time	28 days
Inoculum	Non adapted activated sludge
Test conditions	
Inoculum	Non adapted *activated sludge* from the sewage municipal plant at D-31137 Hildesheim is well suited as it comprises mostly municipal sewage and hardly industrial chemical waste
Pretreatment	The activated sludge was maintained in an aerobic condition by aeration for four hours and then homogenized with a mixer. The sludge was filtered and the filtrate (30 mL) was subsequently used to initiate inoculation.
Test item concentration	30 mg/L, duplicates
Test temperature	20 - 24 °C
Dosing procedure	The test and reference item were weighed in and transferred into the incubation vessels, the vessels made up to 3 L with CO_2 free aqua bidest. and connected to the system for the production of CO_2 free air
Sampling frequency	Backtitration of the residual $Ba(OH)_2$ with 0.05 N HCl was carried out three times a week during the first ten days and thereafter twice weekly. On the day 28 the pH-value of all solutions was measured prior to acidification.
Control	Nutrient solution and inoculum in duplicates

Sampling frequency	Backtitration of the residual $Ba(OH)_2$ with 0.05 N HCl was carried out three times a week during the first ten days and thereafter twice weekly. On the day 28 the pH-value of all solutions was measured prior to acidification.
Control	Nutrient solution and inoculum in duplicates
Functional control	Sodium acetate, 35 mg/L, single
Evaluation	$\text{Degradation [\%]} = \frac{\text{net } CO_2 \cdot 100}{\text{ThCO}_2\ [\text{mgCO}_2/3L]}$
Validity criteria	The study was performed according to OECD 301 B / CO_2 Evolution Test and GLP principles. The validity criteria were fulfilled according to the guideline: The total CO_2 evolution in the control at the end of the test was 46.0 mg/L. The degradation of the functional control reached the pass level of ≥ 60 % by day 14 The degradation of the toxicity control reached the pass level of 35 % after 14 days. The difference of extremes of replicate values of removal of the test item at the end of the test or at the plateau as appropriate was less than 20 %.
Degradation results	The 10 % level (beginning of biodegradation) was reached after an adaptation phase of 5 days The pass level of 60 % was reached after 22 days and the biodegradation came to a maximum of 80 % after 28 days. In the toxicity control a biodegradation rate of 47 % occurred within 14 days and came to a maximum of 70 % after 28 days. The biodegradation of the reference item was not inhibited by the test item in the toxicity control The adaptation phase of the functional control changes after 2 days into the degradation phase (degradation ≥ 10 %) The course of the degradation phase is rapid and reaches a degradation rate > 60 % on day 8. The validity criterion degradation ≥ 60 % after 14 d is fulfilled.

Table 1: **Biodegradation of the Test Item Hostapon TPHC in Comparison to the Functional Control and Toxicity Control**

	Biodegradation [%] study day [d]			
	6	14	21	28
test item, 1st replicate 30 mg/L	23	43	57	82
test item, 2nd replicate 30 mg/L	23	49	61	78
functional control 35 mg/L	48	71	88	100
toxicity control 30 mg/L test item + 35 mg/L reference item	21	47	56	70

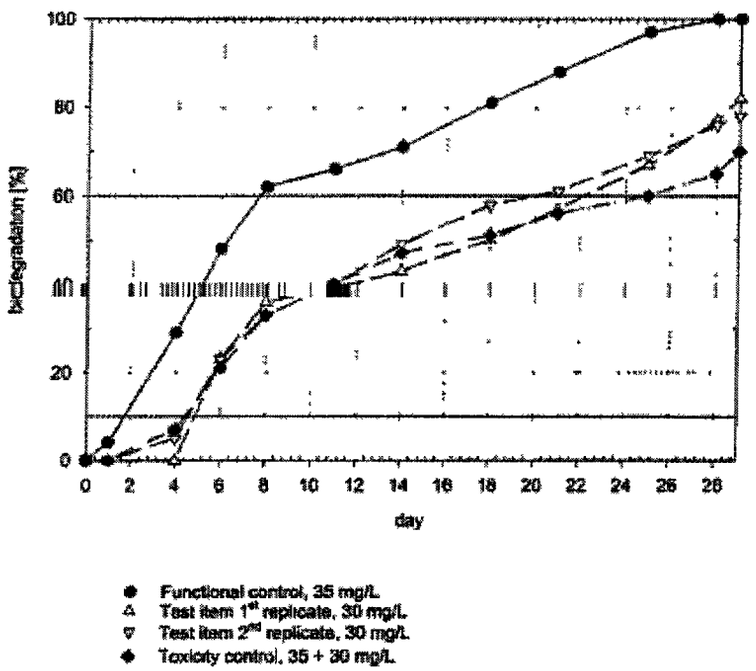

Figure 1. **Biodegradation over a period of 28 days acc. to OECD 301 B**

Conclusions

The test item must be regarded to be readily biodegradable.

References

(1) OECD-Guideline No. 301 for Testing of Chemicals, adopted 1992-07-17

(2) OECD-Guideline No. 301 B for Testing of Chemicals, adopted 1992-07-17

(3) Regulation (EC) No. 648/2004 on Detergents

Reverse Mutation Assay (Ames test) with Salmonella typhimurium

Test Substance: Ethanesulfonic acid, 2-[methyl[(9Z)-1-oxo-9-octadecenyl]amino]-,sodium salt, CAS No. 137-20-2.
Test Substance Purity/Composition: Not specified
Method – OECD 471
Type of Study Reverse Mutation Assay (Ames test) with Salmonella typhimurium
Concentration Range: 0.0016 – 5.0 mg/plate
Year Study Performed 2003
Method/Guideline Followed Yes
GLP Yes
Positive, Negative, and Solvent Control Substance(s) Yes
Species Salmonella typhimurium
Strain TA 97a, TA 98, TA 100, TA 102, and TA 1535
Metabolic Activation: with and without S9 (from male Wistar rats)
Genotoxic Effect Conclusion Not mutagenic in any strain with or without metabolic activation.
Conclusion Not mutagenic
Key Study Sponsor Indicator Clariant
Reference Dr.U. Noack-Laboratorien Study No. USO94302

Hostapon TPHC

Reverse Mutation Assay (Amestest) with *Salmonella typhimurium*

according to
OECD Guideline No. 471 (July, 1997) /
EEC Directive 2000/32/EEC Method, B.13/14. (June, 2000)

Sponsor

CLARIANT GMBH
Div. Surfactants
RQA, C655
D-65926 Frankfurt

Author
S. Fiebig

Testing Facility

DR.U.NOACK-LABORATORIEN
Käthe-Paulus-Str. 1
D-31157 Sarstedt

Laboratory Project ID

Project-No.	030918CL
Study-No.	USO94302
Study-No. (Study plan)	USO9430-

Date
06. Nov. 2003

Original No. 2
of 2 Originals

Statement of GLP Compliance

Title	Hostapon TPHC Reverse Mutation Assay (Amestest) with *Salmonella typhimurium*
Guidelines	OECD Guideline No. 471 (1997) and EEC Directive 2000/32/EEC Method, B.13/14. (2000)
Test Item	Hostapon TPHC (Batch number: DEBD 007534)
Testing Facility	DR.U.NOACK-LABORATORIEN Käthe-Paulus-Str.1, D-31157 Sarstedt Phone: (+49) 050 66 / 706 70, Fax: (+49) 050 66 / 706 789 E-mail: info@noack-lab.de
Deviations from GLP Principles	Purity, content, concentration and storage stability of the test item, respectively were not specified by the sponsor.

We declare that this study was conducted and reported in compliance with the actual OECD principles of Good Laboratory Practice and the national GLP regulations as specified in the law in force, except deviations mentioned above.

6.11.03
(Date)

S. Fiebig
(S. Fiebig, Study Director)

06. Nov. 2003
(Date)

Staatl. anerkannte Prüfeinrichtung DR.U.NOACK-LABORATORIEN gem. § 19b ChemG mit GLP-Zertifikat

(Dr. U. Noack, Head of Testing Facility)

Statement of the Quality Assurance Unit

Title	Hostapon TPHC Reverse Mutation Assay (Amestest) with *Salmonella typhimurium*
Guidelines	OECD Guideline No. 471 (1997) and EEC Directive 2000/32/EEC Method, B.13/14. (2000)
Test Item	Hostapon TPHC (Batch number: DEBD 007534)
Study Director	Silke Fiebig

The study was verified as follows:

inspection	dates	date of report
study plan	2003-10-13	2003-10-13
study based	2003-10-22	2003-10-22
report	2003-11-04 2003-11-06	2003-11-05 2003-11-06

The reported results accurately and completely reflect the raw data of the study. Also methods, procedures, and observations are accurately and completely described in the report.

The accordance of the study with its study plan and the principles of Good Laboratory Practice is guaranteed.

06.11.03 ppa. SBF

Gudrun Möhrmann-Kalabokidis

Personnel Involved

Study Director:	Silke Fiebig (Engineer, Biotechnologist)
Deputy:	Gunda Winkelmann (Engineer of Horticulture)
Technical Staff:	Ute Kutzner Karin Ruthenberg Marlies Schönwälder
Quality Assurance Unit:	Gudrun Möhrmann-Kalabokidis (Biologist)
Deputy:	Susanne Becker (Biologist)
Head of Testing Facility:	Dr. Udo Noack (Biologist)

Table of Content

List of Tables

1 Summary

The mutagenic effects of the test item **Hostapon TPHC** (Batch No. DEBD 007534) were determined in a reverse mutation assay according to OECD Guideline No. 471 and EEC Directive 2000/32/EEC Method B. 13/14 in two independent studies from October 15 to 24, 2003 at DR.U.NOACK-LABORATORIEN in 31157 Sarstedt, Germany. Test systems were the *Salmonella typhimurium* strains TA 97a, TA 98, TA 100, TA 102 and TA 1535 with (+) and without (-) the metabolic activation system S9 (from male Wistar rats) each. Positive and negative controls were included in each study. Duration of each study was 48 h. The test item was dissolved in bidestilled water and applied once at test initiation with the concentration ranges as given in Table 1. Mutagenic and cytotoxic effects are summarized in Table 1.

Table 1: Mutagenic and Cytotoxic Effects of the Test Item

Strain	S9	Tested Concentration Range* [mg/plate]	Lowest Mutagenic Concentration [mg/plate]	Lowest Cytotoxic Concentration [mg/plate]
TA 97a	-	0.0016 - 0.16	none	0.5#
	+	0.016 - 1.6	none	5.0#
TA 98	-	0.05 - 5.0	none	none
	+		none	none
TA 100	-	0.016 - 1.6	none	5.0#
	+		none	5.0#
TA 102	-	0.05 - 5.0	none	none
	+		none	none
TA 1535	-	0.016 - 1.6	none	5.0#
	+		none	5.0#

* = factor √10 # = results of a preliminary test (Non-GLP)

The test item is regarded to be **not mutagenic** under test conditions

2 Characterisation Data of the Test Item

TEST ITEM	Hostapon TPHC
Batch Number	DEBD 007534
Chemical characterisation	Fatty acid methyl tauride, sodium salt
CAS RN	137-20-2
Purity	Not specified
Appearance	Yellowish powder
Solubility in water	150 g/L (20 °C)
Density	Not specified
pH value	7 - 8 (10 g/L, at 20 °C, DIN 53996)
Stability in water	Not specified
Expiry date	2004-03-19 (according to testing facility SOP)
Recommended storage	Room temperature (20 °C)
Storage at test facility	Room temperature, protected from moisture and light
Retention of test item	At least 1 g has been retained.
Identification parameter at testing facility	Name, batch number, state and consistency

The test item and the information concerning the test item were provided by the sponsor.

3 Method

TEST GUIDELINE	OECD Guideline No. 471 for Testing of Chemicals (July, 1997) / EC Directive 2000/32/EC Method B. 13/14. (June, 2000)
TYPE AND PURPOSE OF THE STUDY	Determination of the mutagenic effects of the test item to five different *Salmonella typhimurium* strains over 48 - 72 h.
TEST SYSTEM	*Salmonella typhimurium* strains TA 97a, TA 98, TA 100, TA 102 and TA 1535 with (+) and without (-) metabolic activation system (S9).
Reason for the selection of the test system	*Salmonella typhimurium* strains were selected according to OECD / EC guideline.
Origin	University of California, Berkeley Divison of Biochemistry & Molecular Biology, CA 94720 USA
Storage at test facility	Under liquid nitrogen (≤ -80 °C)
Inoculum	An overnight culture was prepared. Incubation was performed for 18 h at 37 °C. Titer of the overnight culture was ≥ 1×10^8 cells/mL.
Metabolic activation system (S9)	As metabolic activation system a post-mitochondrial (S9) fraction from livers of male Wistar rats, which were induced with Phenobarbital intraperitoneally and β-Naphtoflavone orally was used. The S9 fraction was received from CCR (In den Leppsteinwiesen 19, D-64380 Roßdorf). At test start an aliquot of the S9-fraction was thawed and enriched with cofactors according to DIN Guideline 38415 part 4 (S9-Mix). Batch number of each S9-fraction was 100703 (36.2 mg protein/mL S9).
Media	According to DIN Guideline 38415 Part 4 (December 1999)

Test container

Petri dishes with cams (ø 94 mm, 16 mm high) with about 25 mL VOGEL-BONNER minimal medium (for strain TA 97a with 0.4 % D+ Glucose, for all other strains with 2 % D+ Glucose)

Application / Composition of test media

2.0 mL top agar with 0.5 mM Histidin-/Biotin-solution for plates without metabolic activation system
1.5 mL top agar with 0.5 mM Histidin-/Biotin-solution for plates with metabolic activation system
0.5 mL S9-Mix for plates with metabolic activation system
0.1 mL test item-solution, reference item-solution and aqua bidest. for controls, respectively
0.1 mL bacteria (overnight culture)

Titer control

2.0 mL top agar with 5 mM Histidin- / 0.5 mM Biotin-solution
0.5 mL S9-Mix
0.1 mL bacteria (10^{-5} dilution from overnight culture)

Replicates

Three replicates per concentration level and control.
Titer control replicates were prepared 2-fold.

TEST ITEM

Hostapon TPHC

Test concentrations

Test strain	[mg/plate]
TA 97a - S9	0.0016 - 0.005 - 0.016 - 0.05 - 0.16
TA 97a + S9, TA 100 ± S9, TA 1535 ± S9	0.016 - 0.05 - 0.16 - 0.5 - 1.6
TA 98 ± S9, TA 102 ± S9	0.05 - 0.16 - 0.5 - 1.6 - 5

Stock solution

50 g/L, prepared with aqua bidest.

Dispersion treatment

Agitation

Application

Application was carried out once at test start.

REFERENCE ITEMS

Reference item	Batch No.	*Salmonella* strain / Test Concentration	Purity [%]
ICR 191	40K0600	TA 97a - S9 0.5 µg/plate	99.1
4-Nitro-o-phenylene-diamine	14021CI-070	TA 98 - S9 0.5 µg/plate	98
Nitrofurantoine	98H0515	TA 100 - S9 0.2 µg/plate	< 99
Sodium azide	25692-020	TA 1535 - S9 0.25 µg/Platte	99.6
2-Aminoanthracene	70056-115	TA 97a, TA 98, TA 100, TA 1535 + S9 2 µg/plate	97
Cumene hydroperoxide	91K1681	TA 102 - S9 100 µg/plate	89
Danthron	401821/ 114899	TA 102 + S9 30 µg/plate	98

All reference items are from SIGMA-ALDRICH.

CONTROL

Negative controls were tested with aqua bidest.

TEST METHOD

Confirmation of genotypes

The genotypes of the tested strains were checked at each study by: histidine auxotrophy, ampicillin resistance, tetracycline resistance, UV-sensitivity and growth inhibition by crystal violet.

Temperature (Min-Max)

1. study: 36.4 - 36.7 °C
2. study: 36.7 - 37.0 °C

Test duration	48 h
Experimental dates	1. study: October 15 - 17, 2003 2. study: October 22 - 24, 2003
COURSE OF THE STUDY	Based on the results of a preliminary test (NON-GLP) the test concentrations were selected. Stock solutions were freshly prepared with aqua bidest. each. Inoculum was an overnight culture with a titer ≥ $1 \cdot 10^8$ cells/mL. Two independent studies were conducted as described above. Evaluations were performed as described below.
KIND AND FREQUENCY OF MEASUREMENT AND OBSERVATION	Genotypes were evaluated for each study. Plates of the mutagenicity test were inspected for present and reduced background lawn after incubation. Colonies per plate (revertants) were counted, if no reduced background lawn was observed. Plates with colonies which correspond not with the typical shape and colour of *Salmonella typhimurium* were regarded as contaminated and were not included in calculations.
Equipment	Laboratory incubator, B 6060 (HERAEUS) Microflow biological safety cabinet (MDH) Water bath, W 350 T (MEMMERT) Colony counter (manual counting), BZG 30 (WTW) Thermometer THM912 (HUGER)

EVALUATIONS AND STATISTICS

Since a reduced background lawn is regarded to be a cytotoxic effect, plates with reduced background lawn were not included into evaluation procedures.
Arithmetic mean values and standard deviations were calculated from colonies per plate of three replicates.

For evaluation of the results the induction rate of the mean values was calculated (1):

$$\text{Induction rate} = \frac{\text{revertant colonies of test item}}{\text{revertant colonies of the corresponding control}} \quad (1)$$

The test item is to be interpretated mutagenic if a concentration effect relationship occurred and the induction rate is ≥ 2.

Software

The data presented in the tables are computer generated and rounded for presentation. Thus manual calculation of results based on the data in this report may yield minor deviations from these figures.

Calculations were carried out using software
- Excel 2000 (1985 - 1999), MICROSOFT CORPORATION

VALIDITY CRITERIA

The following genotypes of the tested strains had to be confirmed:
- Histidine auxotrophy
- Ampicillin resistance
- Tetracycline resistance (only TA 102)
- UV-sensitivity (except TA 102)
- Growth inhibition with crystal violet (rfa-mutation)

Titer of the overnight culture had to be ≥ $1 \cdot 10^8$ cells/mL.

Spontaneous revertants/plate (negative controls) should be within the following ranges:
- TA 97 a ± S9: 150 - 450
- TA 98 ± S9: 15 - 50
- TA 100 ± S9: 60 - 200
- TA 102 ± S9: 300 - 600
- TA 1535 ± S9: 5 - 30

The induction rates of the positve controls had to be ≥ 2.

DEVIATIONS FROM THE GUIDELINE

Validity criteria were taken from the literature and DIN guideline, since in OECD and EEC guideline no validity criteria are described.

DEVIATIONS FROM THE STUDY PLAN

None

ARCHIVING

The following will be retained in the archive of the test facility for the period as specified in the operative national GLP regulations:

- all raw data
- study plan (Original 1 of 1)
- final report (Original 1 of 2)
- all records performed by the quality assurance programme including master schedules
- samples of test and reference items

Microfilms will be retained in a safe-deposit by Volksbank Sarstedt, D-31157 Sarstedt.

4 Results

4.1 Test of the Reference Items

Positive controls were tested parallel to the test item.

Table 2: **Induction Rates of Reference Items (Positive Controls)**

Strain	Reference Item	[µg/plate]	S9	Induction Rate	
				1. Study	2. Study
TA 97a	ICR 191	0.5	-	> 4.9	> 4.4
	2-Aminoanthracene	2.0	+	> 5.2	> 4.1
TA 98	4-Nitro-o-phenylenediamine	0.5	-	4.5	3.6
	2-Aminoanthracene	2.0	+	> 40.4	> 39.1
TA 100	Nitrofurantoine	0.2	-	2.4	2.9
	2-Aminoanthracene	2.0	+	> 9.5	> 12.5
TA 102	Cumene hydroperoxide	100.0	-	> 3.0	> 3.4
	Danthron	30.0	+	> 2.5	> 2.6
TA 1535	Sodium azide	0.25	-	6.3	7.7
	2-Aminoanthracene	2.0	+	9.0	8.6

Plates with > 1200 colonies / plate were not counted,
induction rates were calculated with 1200 colonies / plate and given as > values.

4.2 Definitive Tests

Revertant colonies of the test item plates are given in Tables 5 - 24. Spontaneous revertants are listed in Tables 3 - 4.
The arithmetic mean value and the standard deviation were calculated out of the three replicates of the revertant colony plates.
For evaluation of the results the induction rates of the mean values were calculated (Table 5 - 24). Mutagenic and cytotoxic effects are summarized in Table 1.

Table 3: **Spontaneous Revertants (1. Study)**

strain	S9	colonies / plate valid range	counted	mean value	standard deviation
TA 97 a	-	150 - 450	231 248 252	244	11.2
	+		256 230 212	233	22.1
TA 98	-	15 - 50	contaminated 29 29	29	0.0
	+		40 25 24	30	9.0
TA 100	-	60 - 200	116 107 94	106	11.1
	+		118 154 106	126	25.0
TA 102	-	300 - 600	423 367 contaminated	395	39.6
	+		423 500 512	478	48.3
TA 1535	-	5 - 30	24 16 33	24	8.5
	+		10 6 7	8	2.1

contaminated = outlier, not taken into account

Table 4: **Spontaneous Revertants (2. Study)**

strain	S9	colonies / plate valid range	counted	mean value	standard deviation
TA 97 a	-	150 - 450	275 276 267	273	4.9
	+		293 278 302	291	12.1
TA 98	-	15 - 50	26 25 30	27	2.6
	+		35 32 25	31	5.1
TA 100	-	60 - 200	107 100 91	99	8.0
	+		110 97 82	96	14.0
TA 102	-	300 - 600	366 350 351	356	9.0
	+		440 478 446	455	20.4
TA 1535	-	5 - 30	18 20 21	20	1.5
	+		7 9 10	9	1.5

Table 5: **Induction Rates TA 97a - S9 (1. Study)**

test item concentration [mg/plate]	background lawn	colonies / plate	mean value	standard deviation	induction rate
0.16	+	163	194	29.6	0.8
	+	196			
	+	222			
0.05	+	57	247	173.4	1.0
	+	397			
	+	286			
0.016	+	172	206	37.4	0.8
	+	200			
	+	246			
0.005	+	231	253	19.7	1.0
	+	269			
	+	259			
0.0016	+	195	218	32.5	0.9
	+	241			
	+	contaminated			

Table 6: **Induction Rates TA 97a - S9 (2. Study)**

test item concentration [mg/plate]	background lawn	colonies / plate	mean value	standard deviation	induction rate
0.16	+	169	131	71.7	0.5
	+	175			
	+	48			
0.05	+	257	260	6.4	1.0
	+	255			
	+	267			
0.016	+	278	258	18.2	0.9
	+	252			
	+	243			
0.005	+	242	208	33.5	0.8
	+	175			
	+	206			
0.0016	+	214	200	12.4	0.7
	+	193			
	+	192			

\+ = present
\- = reduced
contaminated = outlier, not taken into account

Table 7: **Induction Rates TA 97a + S9 (1. Study)**

test item concentration [mg/plate]	background lawn	colonies / plate	mean value	standard deviation	induction rate
1.6	+	7	54	46.5	0.2
	+	100			
	+	55			
0.5	+	202	181	24.6	0.8
	+	187			
	+	154			
0.16	+	246	243	21.7	1.0
	+	263			
	+	220			
0.05	+	186	212	54.5	0.9
	+	275			
	+	176			
0.016	+	237	211	42.7	0.9
	+	162			
	+	235			

Table 8: **Induction Rates TA 97a + S9 (2. Study)**

test item concentration [mg/plate]	background lawn	colonies / plate	mean value	standard deviation	induction rate
1.6	+	31	58	31.8	0.2
	+	50			
	+	93			
0.5	+	216	199	19.2	0.7
	+	202			
	+	178			
0.16	+	236	241	5.0	0.8
	+	241			
	+	246			
0.05	+	253	255	3.2	0.9
	+	254			
	+	259			
0.016	+	242	216	23.1	0.7
	+	208			
	+	198			

\+ = present
\- = reduced

Table 9: **Induction Rates TA 98 - S9 (1. Study)**

test item concentration [mg/plate]	background lawn	colonies / plate	mean value	standard deviation	induction rate
5	+	34	20	13.6	0.7
	+	18			
	+	7			
1.6	+	9	11	2.1	0.4
	+	12			
	+	contaminated			
0.5	+	18	22	9.6	0.8
	+	15			
	+	33			
0.16	+	98	51	40.6	1.8
	+	24			
	+	32			
0.05	+	17	27	10.0	0.9
	+	37			
	+	27			

Table 10: **Induction Rates TA 98 - S9 (2. Study)**

test item concentration [mg/plate]	background lawn	colonies / plate	mean value	standard deviation	induction rate
5	+	15	15	0.6	0.6
	+	15			
	+	16			
1.6	+	18	16	2.1	0.6
	+	15			
	+	14			
0.5	+	18	19	1.2	0.7
	+	18			
	+	20			
0.16	+	15	21	6.0	0.8
	+	27			
	+	20			
0.05	+	15	21	4.9	0.8
	+	23			
	+	24			

\+ = present
\- = reduced
contaminated = outlier, not taken into account

Table 11: **Induction Rates TA 98 + S9 (1. Study)**

test item concentration [mg/plate]	background lawn	colonies / plate	mean value	standard deviation	induction rate
5	+	18	17	0.6	0.6
	+	17			
	+	17			
1.6	+	15	16	1.7	0.5
	+	18			
	+	15			
0.5	+	31	25	5.3	0.8
	+	21			
	+	23			
0.16	+	38	26	11.5	0.9
	+	26			
	+	15			
0.05	+	40	40	0.6	1.3
	+	39			
	+	40			

Table 12: **Induction Rates TA 98 + S9 (2. Study)**

test item concentration [mg/plate]	background lawn	colonies / plate	mean value	standard deviation	induction rate
5	+	18	18	1.0	0.6
	+	19			
	+	17			
1.6	+	17	23	7.8	0.8
	+	21			
	+	32			
0.5	+	26	25	1.2	0.8
	+	26			
	+	24			
0.16	+	33	31	4.9	1.0
	+	25			
	+	34			
0.05	+	37	38	6.1	1.3
	+	45			
	+	33			

\+ = present
\- = reduced

Table 13: **Induction Rates TA 100 - S9 (1. Study)**

test item concentration [mg/plate]	background lawn	colonies / plate	mean value	standard deviation	induction rate
1.6	+	30	29	1.0	0.3
	+	28			
	+	29			
0.5	+	71	69	2.5	0.6
	+	69			
	+	66			
0.16	+	83	86	6.7	0.8
	+	94			
	+	82			
0.05	+	67	84	18.8	0.8
	+	104			
	+	80			
0.016	+	86	96	11.8	0.9
	+	109			
	+	93			

Table 14: **Induction Rates TA 100 - S9 (2. Study)**

test item concentration [mg/plate]	background lawn	colonies / plate	mean value	standard deviation	induction rate
1.6	+	21	24	3.1	0.2
	+	23			
	+	27			
0.5	+	89	87	4.7	0.9
	+	82			
	+	91			
0.16	+	97	95	6.8	1.0
	+	100			
	+	87			
0.05	+	87	87	18.5	0.9
	+	105			
	+	68			
0.016	+	85	83	2.0	0.8
	+	81			
	+	83			

\+ = present
\- = reduced

Table 15: **Induction Rates TA 100 + S9 (1. Study)**

test item concentration [mg/plate]	background lawn	colonies / plate	mean value	standard deviation	induction rate
1.6	+	65	68	2.5	0.5
	+	68			
	+	70			
0.5	+	79	89	18.8	0.7
	+	111			
	+	78			
0.16	+	94	96	1.5	0.8
	+	97			
	+	96			
0.05	+	118	104	15.8	0.8
	+	108			
	+	87			
0.016	+	96	79	15.3	0.6
	+	76			
	+	66			

Table 16: **Induction Rates TA 100 + S9 (2. Study)**

test item concentration [mg/plate]	background lawn	colonies / plate	mean value	standard deviation	induction rate
1.6	+	61	46	20.0	0.5
	+	23			
	+	53			
0.5	+	104	99	7.8	1.0
	+	103			
	+	90			
0.16	+	69	73	4.5	0.8
	+	73			
	+	78			
0.05	+	64	64	1.0	0.7
	+	63			
	+	65			
0.016	+	66	60	9.5	0.6
	+	65			
	+	49			

\+ = present
\- = reduced

Table 17: **Induction Rates TA 102 - S9 (1. Study)**

test item concentration [mg/plate]	background lawn	colonies / plate	mean value	standard deviation	induction rate
5	+	416	383	37.8	1.0
	+	392			
	+	342			
1.6	+	416	406	15.6	1.0
	+	388			
	+	414			
0.5	+	368	393	36.0	1.0
	+	434			
	+	376			
0.16	+	432	397	41.1	1.0
	+	408			
	+	352			
0.05	+	452	434	25.1	1.1
	+	444			
	+	405			

Table 18: **Induction Rates TA 102 - S9 (2. Study)**

test item concentration [mg/plate]	background lawn	colonies / plate	mean value	standard deviation	induction rate
5	+	305	325	45.4	0.9
	+	377			
	+	293			
1.6	+	313	299	17.2	0.8
	+	305			
	+	280			
0.5	+	307	314	7.0	0.9
	+	313			
	+	321			
0.16	+	308	307	2.1	0.9
	+	305			
	+	309			
0.05	+	359	334	30.7	0.9
	+	344			
	+	300			

\+ = present
\- = reduced

Table 19: **Induction Rates TA 102 + S9 (1. Study)**

test item concentration [mg/plate]	background lawn	colonies / plate	mean value	standard deviation	induction rate
5	+	610	525	131.9	1.1
	+	592			
	+	373			
1.6	+	490	470	18.3	1.0
	+	466			
	+	454			
0.5	+	520	531	81.6	1.1
	+	618			
	+	456			
0.16	+	498	572	65.8	1.2
	+	594			
	+	624			
0.05	+	506	460	103.0	1.0
	+	342			
	+	532			

Table 20: **Induction Rates TA 102 + S9 (2. Study)**

test item concentration [mg/plate]	background lawn	colonies / plate	mean value	standard deviation	induction rate
5	+	432	407	42.7	0.9
	+	358			
	+	432			
1.6	+	388	406	56.6	0.9
	+	360			
	+	469			
0.5	+	590	609	46.9	1.3
	+	574			
	+	662			
0.16	+	540	514	46.8	1.1
	+	460			
	+	542			
0.05	+	440	471	32.0	1.0
	+	470			
	+	504			

\+ = present
\- = reduced

Table 21: **Induction Rates TA 1535 - S9 (1. Study)**

test item concentration [mg/plate]	background lawn	colonies / plate	mean value	standard deviation	induction rate
1.6	+	7	7	2.5	0.3
	+	5			
	+	10			
0.5	+	9	11	2.1	0.5
	+	13			
	+	12			
0.16	+	17	19	2.1	0.8
	+	20			
	+	21			
0.05	+	14	21	7.5	0.9
	+	29			
	+	21			
0.016	+	21	21	1.5	0.8
	+	19			
	+	22			

Table 22: **Induction Rates TA 1535 - S9 (2. Study)**

test item concentration [mg/plate]	background lawn	colonies / plate	mean value	standard deviation	induction rate
1.6	+	6	6	0.6	0.3
	+	5			
	+	6			
0.5	+	20	14	5.2	0.7
	+	11			
	+	11			
0.16	+	19	24	5.6	1.2
	+	23			
	+	30			
0.05	+	28	28	2.0	1.4
	+	30			
	+	26			
0.016	+	16	18	2.1	0.9
	+	20			
	+	19			

\+ = present
\- = reduced

Table 23: **Induction Rates TA 1535 + S9 (1. Study)**

test item concentration [mg/plate]	background lawn	colonies / plate	mean value	standard deviation	induction rate
1.6	+	10	8	1.5	1.1
	+	7			
	+	8			
0.5	+	15	12	3.0	1.6
	+	12			
	+	9			
0.16	+	9	9	0.0	1.2
	+	9			
	+	9			
0.05	+	11	9	1.7	1.2
	+	8			
	+	8			
0.016	+	17	12	4.6	1.6
	+	11			
	+	8			

Table 24: **Induction Rates TA 1535 + S9 (2. Study)**

test item concentration [mg/plate]	background lawn	colonies / plate	mean value	standard deviation	induction rate
1.6	+	6	6	0.6	0.7
	+	6			
	+	5			
0.5	+	5	6	1.5	0.7
	+	6			
	+	8			
0.16	+	11	10	1.7	1.2
	+	11			
	+	8			
0.05	+	12	12	2.0	1.4
	+	10			
	+	14			
0.016	+	12	11	1.2	1.3
	+	12			
	+	10			

\+ = present
\- = reduced

5 Validity Criteria

- The genotypes of the tested strains have been confirmed:
  - Histidine auxotrophy
  - Ampicillin resistance
  - Tetracycline resistance (only TA 102)
  - UV-sensitivity (except TA 102)
  - growth inhibition with crystal violet (rfa-mutation)
- Titer of the overnight cultures was > $1 \cdot 10^8$ cells/mL.
- Spontaneous mutation rates (negative controls) met the requirements.
- The induction rate of the positive controls was ≥ 2.

6 Conclusions

In this study **Hostapon TPHC** was found to have **no mutagenic effects** on *Salmonella typhimurium* strains TA 97 a, TA 98, TA 100, TA 102 and TA 1535 with (+) and without (-) the metabolic activation system S9 from male Wistar rats at non-cytotoxic concentrations. Cytotoxic effects of Hostapon TPHC were determined partly in a preliminary test at concentrations ≥ 0.5 mg/plate. For details see Table 1.

7 Literature / References

(1) OECD Guideline 471 for testing of chemicals: *Salmonella typhimurium*, Reverse Mutation Assay (adopted July 21, 1997)

(2) Richtlinie 2000/32/EG Methode B. 13/14.: Rückmutationsversuch unter Verwendung von Bakterien (Juni 2000)

(3) DIN-Richtlinie 38415 Teil 4 (Dezember 1999)

(4) LEVIN et al. (1982): A new *Salmonella* tester strain (TA 102) with A·T base pairs at the site of mutation detects oxidative mutagens, Proc. Natl. Acad. Sci.. USA, Vol. 79, pp 7445-7449

(5) MARON, D.M. and AMES, B.N. (1983): Revised methods for the Salmonella mutagenicity test, Mutation Research 113, 173-215

8 GLP-Certificate of DR.U.NOACK-LABORATORIUM

Niedersächsisches
Umweltministerium

Gute Laborpraxis/Good Laboratory Practice

GLP-Bescheinigung/Statement of GLP Compliance

(gemäß/according to § 19 b Abs.1 Chemikaliengesetz)

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 88/320/EG wurde durchgeführt in:

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 88/320/EEC at:

X Prüfeinrichtung / Test facility ☐ Prüfstandort / Test site

Dr. U. NOACK LABORATORIUM
für angewandte Biologie
Käthe-Paulus-Str. 1
D-31157 Sarstedt

Dr. U. NOACK LABORATORY
of applied biology
Käthe-Paulus-Str. 1
D-31157 Sarstedt

(Unverwechselbare Bezeichnung und Adresse/Unequivocal name and address)

Prüfungen nach Kategorien/Areas of Expertise
(gemäß/according ChemVwV-GLP Nr. 8.3/OECD guidance)

1 - Prüfungen zur Bestimmung der physikalisch-chemischen Eigenschaften und Gehaltsbestimmungen
3 - Prüfungen zur Bestimmung der erbgutverändernden Eigenschaften (in vitro und in vivo)
4 - Umwelttoxikologische Prüfungen zu Auswirkungen auf aquatische und terrestrische Organismen
5 - Prüfungen zum Verhalten im Boden, im Wasser und in der Luft, Prüfungen zur Bioakkumulation und zur Metabolisierung
6 - Prüfungen zur Bestimmung von Rückständen
7 - Prüfungen zur Bestimmung der Auswirkungen auf Mesokosmen und natürliche Ökosysteme

Datum der Inspektion / Date of Inspection
(Tag.Monat.Jahr / day.month.year)
25. - 27. Juni 2001 / June 25th - 27th, 2001
29. August und 21. September 2001 / August 29th and September 21st, 2001

Die genannte Prüfeinrichtung/Der genannte Prüfstandort befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.

The above mentioned test facility / test-site is included in the national GLP Compliance Programme and is inspected on a regular basis.

Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung/diesem Prüfstandort die oben genannten Prüfungen unter Einhaltung der GLP-Grundsätze durchgeführt werden können.

Based on the inspection report it can be confirmed, that this test facility / test-site is able to conduct the aforementioned studies in compliance with the Principles of GLP.

Niedersächsisches Umweltministerium
Referat 33
Archivstraße 2
30169 Hannover

11. März 2002
Im Auftrage
Dr. Braedt

Robust Summary

Test Item	Hostapon TPHC (batch number: DEBD 007534) Remarks: none
Testing Facility	DR.U.NOACK-LABORATORIEN Käthe-Paulus-Str. 1, D-31157 Sarstedt Tel. +49(0)5066 70670, email: info@noack-lab.de
Author	Silke Fiebig
Report issued	2003-11-06
Method	
Method/Guideline followed	OECD Guideline No. 471 for Testing of Chemicals (July, 1997) / EEC Directive 2000/32/EEC Method B. 13/14. (June, 2000)
Type	Bacterial reverse mutation assay
GLP	Yes [X] No [ ]
Year	2003
Test system	*Salmonella typhimurium* strains TA 97a, TA 98, TA 100, TA 102 and TA 1535
Exposure period	48 h
Metabolic activation	As metabolic activation system a post-mitochondrial (S9) fraction from livers of male Wistar rats, which were induced with Phenobarbital intraperitoneally and β-Naphtoflavone orally was used. At test start an aliquot of the S9-fraction was thawed and enriched with cofactors according to DIN Guideline 38415 part 4 (S9-Mix). Batch number of each S9-fraction was 100703 (36.2 mg protein/mL S9).
Concentrations tested	

Test strain	[mg/plate]
TA 97a - S9	0.0016 - 0.005 - 0.016 - 0.05 - 0.16
TA 97a + S9, TA 100 ± S9, TA 1535 ± S9	0.016 - 0.05 - 0.16 - 0.5 - 1.6
TA 98 ± S9, TA 102 ± S9	0.05 - 0.16 - 0.5 - 1.6 - 5

Statistical Methods	Arithmetic mean values and standard deviations were calculated out of colonies per plate of three replicates. Induction rates of the mean values were calculated out of the relation of revertant colonies of control and test item plates.

Test Conditions

Test Design	Three replicates per concentration level and control. Titer control replicates were prepared 2-fold. Frequency of dosing: Once at test start. Composition of test media: 2.0 mL top agar with 0.5 mM Histidin-/Biotin-solution for plates without metabolic activation system 1.5 mL top agar with 0.5 mM Histidin-/Biotin-solution for plates with metabolic activation system 0.5 mL S9-Mix for plates with metabolic activation system 0.1 mL test item-solution, reference item-solution and aqua bidest. for controls, respectively 0.1 mL bacteria (overnight culture)
Solvent	Aqua bidest.
Description of follow up repeat study	The results were confirmed in two independent studies. Concentrations and test design in the second study were same as in first study.
Criteria for evaluating results	Genotypes were evaluated for each study. Plates of the mutagenicity test were inspected for present and reduced background lawn after incubation. Colonies per plate (revertants) were counted, if no reduced background lawn was observed. Plates with colonies which correspond not with the typical shape and colour of *Salmonella typhimurium* were regarded as contaminated and were not included in calculations.

Results

Table 1: **Mutagenic and Cytotoxic Effects of the Test Item**

Strain	S9	Tested Concentration Range* [mg/plate]	Lowest Mutagenic Concentration [mg/plate]	Lowest Cytotoxic Concentration [mg/plate]
TA 97a	-	0.0016 - 0.16	none	0.5#
	+	0.016 - 1.6	none	5.0#
TA 98	-	0.05 - 5.0	none	none
	+		none	none
TA 100	-	0.016 - 1.6	none	5.0#
	+		none	5.0#
TA 102	-	0.05 - 5.0	none	none
	+		none	none
TA 1535	-	0.016 - 1.6	none	5.0#
	+		none	5.0#

* = factor √10 # = results of a in the preliminary test (Non-GLP)

Conclusion

The test item is regarded to be **not mutagenic** under test conditions.

Contact Hypersensitivity in Guinea Pigs, Buehler Test

Test Substance: Ethanesulfonic acid, 2-[methyl[(9Z)-1-oxo-9-octadecenyl]amino]-,sodium salt, CAS No. 137-20-2.
Test Substance Purity/Composition: 60-65%
Method – OECD 406
Species Albino guinea pigs, Ibm: GOHI: SPF-quality guinea pigs
Route of Induction: Topical
Route of Challenge Exposure Topical
Gender Female
Number of Animals per Dose 20
Concentration 50%
Year Study Performed 2003
Method/Guideline Followed Yes
GLP yes
Exposure Period 6h per treatment
Induction Frequency of Treatment Once per week for 3 weeks
Challenge Exposure Period 6h
Challenge Frequency of Treatment Once, 2 weeks post induction
Total Volume applied and Units 0.5 ml
Control Group Type Positive Control Alpha-Heyxylcinnamaldehyde
Vehicle Used Yes
Vehicle Name PEG 300
Vehicle Amount and Units 50%
Positive Control Substance Alpha-Heyxylcinnamaldehyde
Post-Exposure Period 48 h
Test Results – None of the animals of the control and test group were observed with skin reactions after challenge treatment performed with the highest tested non-irritating concentration of test substance at 50% in PEG 300.
Measurement Period and Units 24 and 48 hrs.
Percent Sensitized Test Substance 0%
Percent Sensitized Positive Control 100%
Percent Sensitized Negative Control 0%
Sensitization Score 0
Conclusion The test substance is not a skin sensitizer
Reliability/Data Quality
Reliability
Reliability Remarks
Key Study Sponsor Indicator Clariant
Reference – RCC Study Number 850718

RCC Study Number 850718

Hostapon TPHC:

Contact Hypersensitivity in Albino Guinea Pigs, Bühler Test

Report

Author: M. Ott

Sponsor: CLARIANT
Dr. Löffler
D 562
Industriepark Höchst
D-65926 Frankfurt
Germany

RCC

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1 PREFACE

1.1 GENERAL

Title	Hostapon TPHC: Contact Hypersensitivity in Albino Guinea Pigs, Bühler Test
Sponsor	CLARIANT Dr. Löffler D 562 Industriepark Höchst D-65926 Frankfurt Germany
Monitoring Scientist	Dr. Kreiling
Test Facility	RCC Ltd Toxicology Operational Unit: Safety Assessment I Wölferstrasse 4 CH-4414 Füllinsdorf / Switzerland

1.2 RESPONSIBILITIES

Study Director	M. Ott
Technical Coordinator	P. Reissbrodt
Head of RCC Quality Assurance	I. Wüthrich

1.3 SCHEDULE

Experimental Starting Date	08-SEP-2003
Experimental Completion Date	16-OCT-2003
Delivery of the Animals	08-SEP-2003
Acclimatization (main study)	08-SEP-2003 to 14-SEP-2003
Observation	08-SEP-2003 to 16-OCT-2003
Treatment (main study)	15-SEP-2003 to 13-OCT-2003
Termination	16-OCT-2003
Study Completion Date	12-NOV-2003

1.4 ARCHIVING

RCC Ltd (CH-4452 Itingen / Switzerland) will retain the study plan, raw data, a sample of test item(s) and the final report of the present study for at least ten years. No data will be discarded without the Sponsor's written consent.

1.5 SIGNATURE PAGE

Study Director: M. Ott

M. Ott

date: 12-NOV-2003

Management: (Hv) Dr. H. Fankhauser

date: 11-NOV-2003

1.6 QUALITY ASSURANCE UNIT

RCC Ltd, Toxicology, CH-4452 Itingen / Switzerland

STATEMENT

RCC STUDY NUMBER : 850718
TEST ITEM : Hostapon TPHC
STUDY DIRECTOR : M. Ott
TITLE : Hostapon TPHC:
Contact Hypersensitivity in Albino Guinea Pigs, Bühler Test

The general facilities and activities are inspected periodically and the results are reported to the responsible person and the management.

Study procedures with exception of the formulation trials were periodically inspected. The study plan and this report were audited by the Quality Assurance Unit. The dates are given below.

Dates and Types of QAU Inspections	Dates of Reports to the Study Director and to Management
03-SEP-2003 Study Plan	03-SEP-2003
08-SEP-2003 Process Based (Test System, Test Item, Treatment, Dose Preparation, Raw Data)	08-SEP-2003
11-NOV-2003 Report	11-NOV-2003

This statement also confirms that this final report reflects the raw data.

Quality Assurance: G. Hohl

.....

date: 12-NOV-2003

GOOD LABORATORY PRACTICE

1.7 STATEMENT OF COMPLIANCE

RCC STUDY NUMBER	:	850718
TEST ITEM	:	Hostapon TPHC
STUDY DIRECTOR	:	M. Ott
TITLE	:	Hostapon TPHC: Contact Hypersensitivity in Albino Guinea Pigs, Bühler Test

The stability of the test item in PEG 300 is unknown. The formulation trials were performed before the study initiation date. Therefore, they are excluded from this statement.

This study has been performed in compliance with the Swiss Ordinance relating to Good Laboratory Practice, adopted February 2nd, 2000 [RS 813.016.5]. This Ordinance is based on the OECD Principles of Good Laboratory Practice, as revised in 1997 and adopted November 26th, 1997 by decision of the OECD Council [C(97)186/Final].

Study Director: M. Ott

M. Ott

.....

date: 12-NOV-2003

1.8 TEST GUIDELINES

The study procedures described in this report meet or exceed the requirements of the following guidelines:

Commission Directive 96/54/EC of 30 July 1996, adapting to technical progress for the 22nd time Council Directive 67/548/EEC. Official journal No. L248, Annex IVC, B.6 "Skin Sensitisation » and Annex V, section 3.2.7.2.

OECD Guidelines for Testing of Chemicals, Number 406 "Skin Sensitization", adopted by the Council on July 17, 1992 (reported Paris, April 29, 1993).

1.9 ANIMAL WELFARE

This study was performed in an AAALAC-approved laboratory in accordance with the Swiss Animal Protection Law under license no. 61.

1.10 CLASSIFICATION GUIDELINES

The evaluation of the results is based on the criteria of the Commission Directive 2001/59/EC of 6 August 2001 adapting to technical progress for the 28th time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. A potential contact sensitizer is classified as any article that produces in a non-adjuvant assay at least 15 % of test animals with allergic contact dermatitis. The test item will be then classified as "may cause sensitization by skin contact" and labelled with the risk phrase R43.

1.11 REFERENCES

Ritz, H.L. and Bühler, E.V.
Current Concepts Cutaneous Toxicity, ed. Drill, V.A. and Lazar, T. (Academic Press, 1980) pp. 25-40: Planning, Conduct and Interpretation of Guinea Pig Sensitization Patch Tests.

2 SUMMARY

The purpose of this skin sensitizing study was to assess the possible allergenic potential of Hostapon TPHC when administered topically to albino guinea pigs.

For this purpose the "Bühler Test" modified by Ritz, H.L. and Bühler, E.V. (1980) was used. Twenty female animals of the test group were treated topically with Hostapon TPHC at 50 % in PEG 300 once a week for a 3-week induction phase. Two weeks after the final induction application the animals were challenged with the same test item concentration of 50 % in PEG 300 as used for induction.

The ten animals of the control group were not treated during the induction. They were treated once at challenge with Hostapon TPHC at 50 % in PEG 300.

Results

None of the control and test animals were observed with skin reactions after the challenge treatment with the highest tested non-irritating concentration of Hostapon TPHC at 50 % in PEG 300.

PRIMARY SENSITIZATION RESULTS (INCIDENCE TABLES)

CHALLENGE

The highest tested non-irritating concentration of Hostapon TPHC used for challenge was 50 % in PEG 300. The incidence of positive erythema reactions after topical challenge is described as follows:

ERYTHEMA SCORE	TEST GROUP 20 animals		CONTROL GROUP 10 animals	
	24 hrs	48 hrs	24 hrs	48 hrs
0	20	20	10	10
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
No. with grades ≥ 1	0	0	0	0
No. tested	20	20	10	10
INCIDENCE*	0/20		0/10	
SEVERITY**	0		0	

* Number of animals showing a response of grade 1 or greater at either 24- or 48-hour reading out of the total animals.

** Total sum of 24- and 48-hour response readings divided by the number of animals exposed (maximum of 3).

3 CONCLUSION

In this study none of the animals of the control and test group were observed with skin reactions after challenge treatment performed with the highest tested non-irritating concentration of Hostapon TPHC at 50 % in PEG 300.

Based on the above mentioned findings in a non-adjuvant sensitization test in guinea pigs and in accordance to Commission Directive 2001/59/EC, Hostapon TPHC applied at a concentration of 50 % in PEG 300 does not have to be classified and labelled as a skin sensitizer.

4 PURPOSE

The purpose of this skin sensitization study was to determine if the test item under the conditions described in the study plan and this report, causes an increased reaction in the skin of guinea pigs at challenge when compared to appropriate controls.

This study should provide a rational basis for risk assessment of the sensitizing potential of the test item in man.

The sensitivity and reliability of the experimental technique employed was assessed by use of ALPHA-HEXYLCINNAMALDEHYDE which is recommended by the OECD 406 Guidelines and is known to have moderate skin sensitization properties in the guinea pig strain. The results from the most recent test run (RCC study number 848094, performed from 01-APR-2003 to 08-MAY-2003) are included in this report under the APPENDIX E.

5 MATERIALS AND METHODS

5.1 TEST SYSTEM

Test system	Ibm: GOHI; SPF-quality guinea pigs (synonym: Himalayan spotted)
Rationale	Skin reactions in the guinea pig are classically used for determining the potential of test items to induce delayed contact hypersensitivity. No valid non-animal model (*in-vitro*) is available at present for the test of contact sensitization.
Source	RCC Ltd, Laboratory Animal Services CH-4414 Füllinsdorf / Switzerland
Number of animals for main study / Irritation screen	30 females / 4 females (nulliparous and non-pregnant) Challenge: - 20 test animals - 10 control animals Irritation Screen: - 4 animals
Age at delivery/ acclimatization start	4 - 6 weeks
Body weight at delivery/ acclimatization start	Test and control animals: 328 - 422 g Animals used for irritation screen: 339 - 369 g
Identification	By unique cage number and corresponding ear tags.
Randomization	Randomly selected by hand at time of delivery. No computer randomization.

Acclimatization

Under test conditions after health examination. One week for the control and test group. However, contrary to the test group the control group remained untreated during the 3 induction weeks.
One day for the animals used in the irritation screen for induction and challenge. Only animals without any visible signs of illness were used for the study.

5.2 ALLOCATION

The animals were distributed as follows:

	NUMBER OF ANIMALS PER GROUP	ANIMAL NUMBERS PER GROUP
1 Irritation Screen for Induction and Challenge	4	801 - 804
2 Control Group	10	805 - 814
3 Test Group	20	815 - 834

5.3 HUSBANDRY

Room no.

105 / RCC Ltd, Füllinsdorf

Conditions

Standard Laboratory Conditions
Air-conditioned with target ranges for room temperature 20 ± 3 °C, relative humidity 30-70 % and approximately 10-15 air changes per hour. Room temperature and humidity were monitored continuously and values outside of these ranges occasionally occurred, usually following room cleaning. These transient variations are considered not to have any influence on the study and, therefore, these data are not reported but are retained at RCC. The animals were provided with an automatically controlled light cycle of 12 hours light and 12 hours dark. Music was played during the daytime light period.

Accommodation

Individually in Makrolon type-4 cages with standard softwood bedding ("Lignocel", Schill AG, CH-4132 Muttenz).

Diet

Pelleted standard Provimi Kliba 3418, batch nos. 43/03 and 52/03, guinea pig breeding / maintenance diet, containing Vitamin C (Provimi Kliba AG, CH-4303 Kaiseraugst), *ad libitum*. Results of analyses for contaminants are archived at RCC Ltd, Itingen.

Water

Community tap water from Füllinsdorf, *ad libitum*. Results of bacteriological, chemical and contaminant analyses are archived at RCC Ltd, Itingen.

5.4 TEST ITEM

The following information was provided by the sponsor:

Identification	Hostapon TPHC
Description	Yellow powder
Batch number	DEBD 007534
Concentration	60 – 65 %
Stability of test item	Stable under storage conditions; expiration date: April 2005
Stability of test item dilution	Unknown in PEG 300; is excluded from the statement of compliance.
Storage conditions	At room temperature (range of 20 ± 3 °C), protected from light.
Safety precautions	Routine hygienic procedures were used to ensure the health and safety of the personnel.

5.5 VEHICLE

The following information was provided by RCC Ltd:

Identification	Polyethylene glycol 300 (PEG 300)
Description	Colorless viscous liquid
Lot number	448174/1 21203148
Source	FLUKA Chemie GmbH, CH-9471 Buchs
Stability of vehicle	Stable under storage conditions; expiration date: 16-APR-2005
Storage conditions	At room temperature (range of 20 ± 3 °C), protected from light.
Safety precautions	Routine hygienic procedures were used to ensure the health and safety of the personnel.

The vehicle was selected based on preliminary solubility testing which was performed before the study initiation date. Therefore, the formulation trials were excluded from the statement of GLP compliance. PEG 300 was a suitable vehicle to be used for the study.

5.6 TEST ITEM PREPARATION

The test item and vehicle were placed into a glass beaker on a tared Mettler PM 460 balance and weight/weight dilutions were prepared. Homogeneity of the test item in PEG 300 was ensured and maintained during treatment using a magnetic stirrer and/or spatula. The preparations were made immediately prior to each dosing.

Dose levels were in terms of material as supplied by the sponsor.

5.7 RATIONALE

The dermal route has historically been used as the route of choice for determining delayed contact hypersensitivity.

5.8 SELECTION OF CONCENTRATION OF TEST ITEM FOR MAIN STUDY

A number of factors contributed to the selection of the concentrations of test item including irritancy, slope of dose response curve and experience with similar test items. Selection was based on the following criteria:

Epidermal Induction: Concentration that produced some irritation but not adversely affected the animals (determined at the irritation screen). In this study, the highest technically applicable concentration did not produce any skin reaction.

Epidermal Challenge: Concentration that was the maximum tested non-irritant concentration.

5.9 GRADING METHOD

The test item skin area of the animals used for irritation screen and challenge were depilated 21 hours after the patches had been removed, using an approved depilatory cream (VEET Cream, Reckitt & Colman AG, CH-4123 Allschwil). The depilation was performed to facilitate the reading of the skin reactions. The depilatory cream was placed on the patch sites and surrounding areas, and left on for up to 3-5 minutes. It was then thoroughly washed off with a stream of warm, running water. The animals were then dried with a disposable towel, and returned to their cages.

The scoring system was performed by visual assessment of erythema, oedema and other clinical changes in skin conditions. They were assessed as follows:

0 = no visible change
1 = discrete or patchy erythema
2 = moderate and confluent erythema
3 = intense erythema and swelling

Grading of all animals was done by positioning each animal under true-light (Philips TLD 36W/84 or Osram 36W/31 830).

The grading method used for irritation screen, induction and challenge was identical. It was performed 24 hours after removal of the patches for irritation screen, induction and challenge and repeated 24 hours later (48-hour grades) for the irritation screen and the challenge.

Note: At challenge, control animals were graded before the test animals.

5.10 TREATMENT METHODS

Patching method: The same patching method was used for irritation screen, induction and challenge.

The animal's fur was shaved with a fine clipper blade just prior to the exposure. Closed patches were applied to the animals as follows:

0.5 mL of the freshly prepared test item solution in a 25 mm Hill Top Chamber.

The 25 mm Hill Top Chamber was firmly secured by an elastic plaster wrapped around the trunk of the animal and secured with impervious adhesive tape. The occlusive dressing was left in place for six hours (± 15 minutes).

6 STUDY CONDUCT - TREATMENT PROCEDURE

6.1 DIAGRAMMATIC STUDY PLAN

Acclimatization		Study day				
-7	-6	1	8	15	22	29
	IS	I	I	I		C

IS = Irritation screen to determine the minimal irritating concentration used in the induction period and the highest non-irritating concentration used for the challenge.
I = Induction (test group only)
C = Challenge (control and test group)

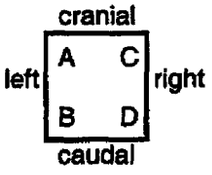
6.2 IRRITATION SCREEN FOR INDUCTION AND CHALLENGE – PERFORMED DURING THE ACCLIMATIZATION PERIOD

The test item concentrations described below were selected during a preliminary solubility testing which was performed before the study initiation date.

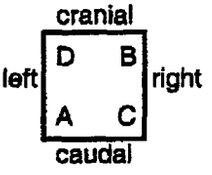
For patch placements, the format described below was used on 4 guinea pigs. Four different concentrations were used on each animal for a 6-hour exposure period.

The test item concentration of 50 % in PEG 300 was considered to be the most qualified to assure an optimum technical application procedure.

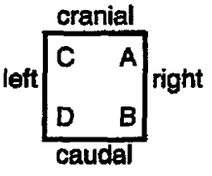
Test Item	Concentrations		Vehicle	Formulation
Hostapon TPHC	A = 50 % B = 25 %	C = 15 % D = 10 %	PEG 300	weight/weight



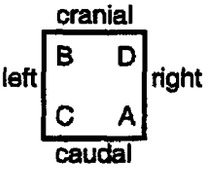
Animal no. 801



Animal no. 802



Animal no. 803



Animal no. 804

The allocation of the different test item dilutions to the sites (A, B, C, D) on the four animals was alternated in order to minimize site-to-site variation in responsiveness.

The application sites were assessed for erythema and oedema 24 and 48 hours after removal of the patches.

The results are described on page 23 and are summarized as follows:

	Irritancy Results							
	after the 24-hour reading concentration (%) of Hostapon TPHC				after the 48-hour reading concentration (%) of Hostapon TPHC			
Response Grade	50 %	25 %	15 %	10 %	50 %	25 %	15 %	10 %
0	4*	4	4	4	4	4	4	4
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0

* = number of grade-related skin response

The most representative concentration to stimulate a state of immune hypersensitivity was 50 % used in the induction phase and also used in the challenge as the highest non-irritating concentration.

6.3 INDUCTION

The concentration of the test item required for the induction was agreed between the Study Director and responsible Technical Coordinator after the irritation screen had been completed.

The fur was clipped from the left shoulder of each test animal and the patches applied, over a period of 3 weeks. Each animal received one patch per week with the test item at 50 % in PEG 300 which remained in place for approximately 6 hours each. The repeated application was performed at the same site. The interval between exposure was one week. The control animals remained untreated.

After the last induction exposure the test animals were left untreated for 2 weeks before the challenge.

The skin responses were graded 24 hours after the patches had been removed.

Any gross skin reactions were recorded without depilation.

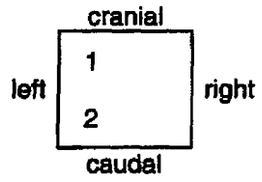
6.4 CHALLENGE – PERFORMED ON TEST DAY 29

The concentration of the test item required for the challenge was agreed between the Study Director and responsible Technical Coordinator after the irritation screen had been completed.

The animals previously exposed during the induction period (i.e. test group) as well as the previously untreated control animals were challenged two weeks after the last induction exposure using the test item at 50 % in PEG 300. The fur was clipped from the left posterior quadrant of the side and back of the animals. Patch sites for challenge are indicated below. The exposure period was 6 hours on a naive skin site.

The responses were graded at 24 and 48 hours after the patches had been removed, according to the grading method described above.

6.5 FORMAT FOR INDUCTION AND CHALLENGE PATCH APPLICATION



1 = Induction (test group only)
2 = Challenge (control and test group)

6.6 OBSERVATIONS

The following observations and data were recorded during the study:

Viability / Mortality	Daily from delivery of the animals to the termination of test.
Clinical signs / Grading of skin response score	Daily from delivery of the animals to the termination of test. Skin responses were graded during the irritation screen, induction and challenge period.
Body weights	At acclimatization and treatment start, and at the termination of the study.

Records were maintained of all additional and standard observations.

These observations applied to the main study groups and to the irritation screen group to the extent of their use in the study.

6.7 EVALUATION OF SKIN REACTIONS

For evaluation, two parameters were used: the incidence index and the severity index, for both test and control animals. The incidence index is an expression of the number of animals showing a response of grade 1 or greater at either 24- or 48-hour reading out of the total animals in the group, while the severity index is calculated from the total sum of 24- and 48-hour response readings divided by the number of animals exposed.

In this study, the incidence and severity index are of zero.

7 PATHOLOGY

7.1 NECROPSY

No necropsies were performed on the animals of the control and test group sacrificed at termination of their observation period or on the animals of the irritation screen sacrificed on test day 1.

The animals were euthanized by intraperitoneal injection of Vetanarcol at a dose of at least 2.0 mL/kg body weight (equivalent to 324 mg sodium pentobarbitone/kg body weight) and discarded.

8 STATISTICAL ANALYSIS

Descriptive statistics (means and standard deviations) were calculated for body weights. No inferential statistics were used.

9 DATA COMPILATION

The following data were recorded on data sheets and transcribed for compilation and analysis: skin reactions, viability/mortality, clinical signs.

The following data were recorded on-line: body weights.

10 RESULTS
Main Study

10.1 VIABILITY / MORTALITY / MACROSCOPIC FINDINGS

There were no deaths during the course of the study, hence no necropsies were performed.

10.2 CLINICAL SIGNS, SYSTEMIC

No symptoms of systemic toxicity were observed in the animals.

10.3 SKIN EFFECT IN THE INDUCTION

No skin effect was observed in the three weeks of induction.

See p. 25

10.4 SKIN EFFECT IN THE CHALLENGE

No skin reactions were observed in the control and test animals treated with the test item at 50 % in PEG 300.

The control and test animals were depilated 3 hours prior to the 24-hour reading to facilitate the reading of the skin reactions.

See p. 27

10.5 BODY WEIGHTS

The body weight of the animals was within the range commonly recorded for animals of this strain and age.

See pp. 29 - 31

APPENDIX A

SKIN REACTIONS DURING IRRITATION SCREEN FOR INDUCTION AND CHALLENGE

- INDIVIDUAL FINDINGS

SKIN REACTIONS DURING IRRITATION SCREEN FOR INDUCTION AND CHALLENGE – INDIVIDUAL FINDINGS

IRRITATION SCREEN

Animal No.: 801 female

	Skin reactions after	
	24 Hours	48 Hours
A = 50 %	0	0
B = 25 %	0	0

	Skin reactions after	
	24 Hours	48 Hours
C = 15 %	0	0
D = 10 %	0	0

Animal No.: 802 female

	Skin reactions after	
	24 Hours	48 Hours
D = 10 %	0	0
A = 50 %	0	0

	Skin reactions after	
	24 Hours	48 Hours
B = 25 %	0	0
C = 15 %	0	0

Animal No.: 803 female

	Skin reactions after	
	24 Hours	48 Hours
C = 15 %	0	0
D = 10 %	0	0

	Skin reactions after	
	24 Hours	48 Hours
A = 50 %	0	0
B = 25 %	0	0

Animal No.: 804 female

	Skin reactions after	
	24 Hours	48 Hours
B = 25 %	0	0
C = 15 %	0	0

	Skin reactions after	
	24 Hours	48 Hours
D = 10 %	0	0
A = 50 %	0	0

Three hours prior to the 24-hour reading both flanks were depilated.

APPENDIX B

SKIN REACTIONS OBSERVED DURING INDUCTION

- INDIVIDUAL FINDINGS

SKIN REACTIONS OBSERVED DURING INDUCTION – INDIVIDUAL FINDINGS

INDUCTION WEEK 1 / application on test day 1

Test item concentration: 50 %
Vehicle: PEG 300

TEST GROUP

Animal number female	815	816	817	818	819	820	821	822	823	824
Skin reaction	0	0	0	0	0	0	0	0	0	0

Animal number female	825	826	827	828	829	830	831	832	833	834
Skin reaction	0	0	0	0	0	0	0	0	0	0

INDUCTION WEEK 2 / application on test day 8

Test item concentration: 50 %
Vehicle: PEG 300

TEST GROUP

Animal number female	815	816	817	818	819	820	821	822	823	824
Skin reaction	0	0	0	0	0	0	0	0	0	0

Animal number female	825	826	827	828	829	830	831	832	833	834
Skin reaction	0	0	0	0	0	0	0	0	0	0

INDUCTION WEEK 3 / application on test day 15

Test item concentration: 50 %
Vehicle: PEG 300

TEST GROUP

Animal number female	815	816	817	818	819	820	821	822	823	824
Skin reaction	0	0	0	0	0	0	0	0	0	0

Animal number female	825	826	827	828	829	830	831	832	833	834
Skin reaction	0	0	0	0	0	0	0	0	0	0

APPENDIX C

SKIN REACTIONS AFTER CHALLENGE

- INDIVIDUAL FINDINGS

SKIN REACTIONS AFTER CHALLENGE – INDIVIDUAL FINDINGS

Test item: Hostapon TPHC

Test item concentration: 50 %

Vehicle: PEG 300

CONTROL GROUP

Animal No. female	Skin Reactions after (± 2 Hours) 24 Hours	48 Hours
805	0	0
806	0	0
807	0	0
808	0	0
809	0	0

Animal No. female	Skin Reactions after (± 2 Hours) 24 Hours	48 Hours
810	0	0
811	0	0
812	0	0
813	0	0
814	0	0

TEST GROUP

Animal No. female	Skin Reactions after (± 2 Hours) 24 Hours	48 Hours
815	0	0
816	0	0
817	0	0
818	0	0
819	0	0
820	0	0
821	0	0
822	0	0
823	0	0
824	0	0

Animal No. female	Skin Reactions after (± 2 Hours) 24 Hours	48 Hours
825	0	0
826	0	0
827	0	0
828	0	0
829	0	0
830	0	0
831	0	0
832	0	0
833	0	0
834	0	0

Three hours prior to the 24-hour reading, the test item-treated flank was depilated.

APPENDIX D

BODY WEIGHTS

- SUMMARY

- INDIVIDUAL

BODY WEIGHTS – SUMMARY

BODY WEIGHTS (GRAM) SUMMARY
FEMALES

ACCLIMATIAZTION		GROUP 1 IRRITATION SCREEN	GROUP 2 CONTROL GROUP	GROUP 3 TEST GROUP
DAY 1	MEAN	356	358	365
WEEK 1	ST.DEV.	13.5	17.5	23.9
	MINIMUM	339	335	328
	MAXIMUM	369	384	422
	N	4	10	20

BODY WEIGHTS – SUMMARY (CONTINUED)

BODY WEIGHTS (GRAM) SUMMARY
FEMALES

TREATMENT			GROUP 1 IRRITATION SCREEN	GROUP 2 CONTROL GROUP	GROUP 3 TEST GROUP
DAY	1	MEAN	369	381	397
WEEK	1	ST.DEV.	16.5	19.3	21.6
		MINIMUM	355	347	361
		MAXIMUM	393	413	440
		N	4	10	20
DAY	32	MEAN	---	512	523
WEEK	5	ST.DEV.	---	45.1	39.0
		MINIMUM	---	450	405
		MAXIMUM	---	587	597
		N	0	10	20

BODY WEIGHTS – INDIVIDUAL

BODY WEIGHTS (GRAM)
FEMALES

	ACCLIMATIAZTION	TREATMENT	
DAYS	1	1	32
WEEKS	1	1	5
ANIMAL			
GROUP 1 (IRRITATION SCREEN)			
801	339	355	---
802	364	364	---
803	369	393	---
804	352	363	---
GROUP 2 (CONTROL GROUP)			
805	362	378	540
806	341	347	450
807	384	413	571
808	364	391	482
809	369	386	491
810	340	377	461
811	374	398	492
812	371	367	587
813	335	359	526
814	340	389	523
GROUP 3 (TEST GROUP)			
815	345	381	495
816	388	436	597
817	339	368	486
818	366	386	510
819	349	371	511
820	378	416	583
821	339	394	520
822	422	431	546
823	407	440	555
824	376	398	533
825	355	400	513
826	343	361	522
827	361	409	508
828	367	389	517
829	346	377	527
830	328	394	538
831	378	392	496
832	376	404	561
833	355	392	405
834	379	403	565

APPENDIX E

RESULTS OF POSITIVE CONTROL

RESULTS OF POSITIVE CONTROL

RCC Study Number 848094

ALPHA-HEXYLCINNAMALDEHYDE:

Contact Hypersensitivity in Albino Guinea Pigs, Bühler Test

POSITIVE CONTROL

performed from 01-APR-2003 to 08-MAY-2003

RESULTS OF POSITIVE CONTROL (CONTINUED)

SUMMARY

For validation of the sensitivity of test method and test system used, a known moderate sensitizer ALPHA-HEXYLCINNAMALDEHYDE was selected as a positive control. This was performed from 01-APR-2003 to 08-MAY-2003 in accordance with the recommendation of:

OECD Guidelines for Testing of Chemicals, Number 406 "Skin Sensitization", adopted by the Council on July 17, 1992 (reported Paris, April 29, 1993).

The raw data from this study are kept in a separate file at RCC Ltd, CH-4452 Itingen. The test described was performed under GLP-conditions with a final QA-check.

TEST ITEM

Identification	ALPHA-HEXYLCINNAMALDEHYDE
Description	yellow liquid
Date of test item receipt	07-SEP-2001
Lot number	01016AQ
Purity	85 %
Stability of test item	Stable under storage conditions; expiration date: 07-SEP-2004
Stability of test item dilution	Stable in PEG 300 for at least 2 hours at room temperature (determined at RCC Ltd, Environmental Chemistry & Pharmanalytics Division, under non-GLP conditions).
Storage conditions	In the original container, at room temperature (range of 20 ± 3 °C), away from direct sunlight.
Safety precautions	Routine hygienic procedures were used to ensure the health and safety of the personnel.

RESULTS OF POSITIVE CONTROL (CONTINUED)

VEHICLE

Identification	Polyethylene glycol 300 (PEG 300)
Description	colorless viscous liquid
Lot number	442989/1 54502013
Source	FLUKA Chemie GmbH, CH-9471 Buchs
Stability of vehicle	Stable under storage conditions; expiration date: 14-NOV-2003
Storage conditions	In the original container, at room temperature (range of 20 ± 3 °C), away from direct sunlight.
Safety precautions	Routine hygienic procedures were used to ensure the health and safety of the personnel.

TEST SYSTEM

Test system	Ibm: GOHI; SPF-quality guinea pigs (synonym: Himalayan spotted)
Rationale	Skin reactions in the guinea pig are classically used for determining the potential of test items to induce delayed contact hypersensitivity. No valid non-animal model (*in-vitro*) is available at present for the test of contact sensitization.
Source	RCC Ltd, Laboratory Animal Services CH-4414 Füllinsdorf / Switzerland
Number of animals for main study / Irritation screen	30 males / 4 males Challenge: - 20 test animals - 10 control animals Irritation Screen: - 4 animals
Age at delivery/ acclimatization start	5 - 7 weeks
Body weight at delivery/ acclimatization start	Test and control animals: 388 - 444 g Animals used for irritation screen: 386 - 431 g
Identification	By unique cage number and corresponding ear tags.
Randomization	Randomly selected by hand at time of delivery. No computer randomization.

RESULTS OF POSITIVE CONTROL (CONTINUED)

Acclimatization	Under test conditions after health examination. One week for the control and test group. However, contrary to the test group the control group remained untreated during the 3 induction weeks. One day for the animals used in the irritation screen for induction and challenge. Only animals without any visible signs of illness were used for the study.

The purpose of this skin sensitizing study was to confirm the possible allergenic potential of ALPHA-HEXYLCINNAMALDEHYDE and to prove the sensitivity of the test system when administered topically to albino guinea pigs.

For this purpose the "Bühler Test" modified by Ritz, H.L. and Bühler, E.V. (1980) was used. Twenty male animals of the test group were treated topically with ALPHA-HEXYLCINNAMALDEHYDE at 50 % in PEG 300 once a week for a 3 week induction phase. Two weeks after the final induction application the animals were challenged with the test item concentration of 5 % in PEG 300.
The ten animals of the control group were not treated during the induction. They were treated once at challenge with ALPHA-HEXYLCINNAMALDEHYDE at 5 % in PEG 300.

Results

Twenty (at the 24-hour reading) and nineteen (at the 48-hour reading) out of 20 test animals were observed with discrete/patchy to moderate/confluent erythema after the challenge treatment with the highest tested non-irritating concentration of ALPHA-HEXYLCINNAMALDEHYDE at 5 % in PEG 300. No skin effect was observed in the control group.

RESULTS OF POSITIVE CONTROL (CONTINUED)

PRIMARY SENSITIZATION RESULTS (INCIDENCE TABLES)

CHALLENGE

The highest tested non-irritating concentration of ALPHA-HEXYLCINNAMALDEHYDE used for challenge was 5 % in PEG 300. The incidence of positive erythema reactions after topical challenge is described as follows:

ERYTHEMA SCORE	TEST GROUP 20 animals		CONTROL GROUP 10 animals	
	24 hrs	48 hrs	24 hrs	48 hrs
0	0	1	10	10
1	13	14	0	0
2	7	5	0	0
3	0	0	0	0
No. with grades ≥ 1	20	19	0	0
No. tested	20	20	10	10
INCIDENCE*	20/20		0/10	
SEVERITY**	1.2 – 1.35		0	

* Number of animals showing a response of grade 1 or greater at either 24- or 48-hour reading out of the total animals.

** Total sum of 24- and 48-hour response readings divided by the number of animals exposed (maximum of 3).

CONCLUSION

In this study 100 % (at the 24-hour reading) of the animals of the test group were observed with skin reactions after challenge treatment performed with the highest tested non-irritating concentration of ALPHA-HEXYLCINNAMALDEHYDE at 5 % in PEG 300.

No skin reactions were observed in the control group treated in the same conditions during the challenge phase.

Based on the above mentioned findings in a non-adjuvant sensitization test in guinea pigs and in accordance to Commission Directive 96/54/EEC, ALPHA-HEXYLCINNAMALDEHYDE applied at a concentration of 5 % in PEG 300 does have to be classified and labelled as a skin sensitizer and proved the sensitivity of the test system.

RESULTS OF POSITIVE CONTROL (CONTINUED)

CHALLENGE

Test item: ALPHA-HEXYLCINNAMALDEHYDE

Test item concentration: 5 %

Vehicle: PEG 300

CONTROL GROUP

Animal No. male	Skin Reactions after (± 2 Hours) 24 Hours	48 Hours
847	0	0
848	0	0
849	0	0
850	0	0
851	0	0

Animal No. male	Skin Reactions after (± 2 Hours) 24 Hours	48 Hours
852	0	0
853	0	0
854	0	0
855	0	0
856	0	0

TEST GROUP

Animal No. male	Skin Reactions after (± 2 Hours) 24 Hours	48 Hours
857	1	1
858	1	1
859	1	1
860	2	2
861	2	1
862	1	1
863	1	1
864	2	1
865	1	1
866	2	2

Animal No. male	Skin Reactions after (± 2 Hours) 24 Hours	48 Hours
867	1	1
868	1	1
869	2	2
870	2	2
871	2	2
872	1	1
873	1	1
874	1	0
875	1	1
876	1	1

Three hours prior to the 24-hour reading, the test item-treated flank was depilated.

APPENDIX F

SUMMARY TABLE OF STUDY INFORMATION AND RESULTS

SUMMARY TABLE OF STUDY INFORMATION AND RESULTS

Test item identification: Hostapon TPHC				
SKIN TOLERANCE STUDIES / IMMUNOSTIMULATION (SENSITIZATION POTENTIAL BY EPICUTANEOUS ADMINISTRATION - BÜHLER TEST)				
Batch No.: DEBD 007534 RCC Study No.: 850718 Study Completion Date: 12-NOV-2003				
Species/Strain: Ibm: GOHI; SPF-quality guinea pigs (synonym: Himalayan spotted)			Number of exposed animals: 30	
Procedure	Administration route/site		Day	Vehicle
Induction phase/ 6-hour application	Occl. patch/left shoulder		1, 8, 15	PEG 300
Challenge/ 6-hour application	Occl. patch/left flank		29	PEG 300
Study Group	Control Group		Test Group	
	Concentration of test item	Number of appl. and dose	Concentration of test item	Number of appl. and dose
Induction phase/ 6-hour application	---	---	50 %	1x0.5mL/week/ 25mm Hill Top Chamber
Challenge/ 6-hour application	50 %	1x0.5mL/25mm Hill Top Chamber	50 %	1x0.5mL/25mm Hill Top Chamber
Number of animals and sex	10 females		20 females	
Number of animals showing a grade ≥ 1 at either 24 or 48 hours / out of total (incidence index)				
Challenge		0/10	0/20	
Summary of salient findings: The test item tested under the described conditions is considered not to be a skin sensitizer.				
Study in compliance with GLP:			YES: X	NO:
QA inspected/audited:			YES: X	NO:

APPENDIX G

GLP – CERTIFICATION

GLP – CERTIFICATION

The Swiss GLP Monitoring Authorities

Swiss Federal Office of Public Health

Swiss Agency for the Environment, Forests and Landscape

swissmedic Swissmedic Swiss Agency for Therapeutic Products

Statement of GLP Compliance

It is hereby confirmed that

during the period of — November 18 – 22, 2002

the following Test Facilities of — **RCC Ltd**
4452 Itingen
Switzerland

were inspected by the Federal Office of Public Health, the Swiss Agency for Therapeutic Products and the Swiss Agency for the Environment, Forests and Landscape with respect to the compliance with the Swiss legislation on Good Laboratory Practice.

Test Facilities	Areas of expertise *
- Toxicology	TOX, ACC, MUT, OTH (Safety Pharmacology)
- Environmental Chemistry and Pharmanalytics	ACC, ECT, ENF, EMN, PCT, RES, OTH (Animal metabolism)

The inspections were performed in agreement with the OECD Guidelines for National GLP Inspections and Audits. It was found that the aforementioned test facilities were operating in compliance with the Swiss Ordinance relating to Good Laboratory Practice [RS 813.016.5] at the time they were inspected.

Federal Office of Public Health
The Director

Bern, March 2003

Prof. Th. Zeltner

* TOX = Toxicology ; ACC = Analytical and Clinical Chemistry ; ECT = Environmental toxicity on aquatic and terrestrial organisms ; ENF = Behaviour in water, soil and air. Bioaccumulation ; EMN = Studies on effects on mesocosms and natural ecosystems; MUT = Mutagenicity; PCT = Physical-chemical testing ; RES = Residue studies ; OTH = Other, to be specified.